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THE BLOOD SUPPLY OF NERVES

I. HISTORICAL REVIEW

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Since Isenflamm & Doerffler's original work on the vasa nervorum (1768) there have appeared in the literature frequent references to the possible importance of these vessels in the causation of various peripheral nervous disorders. During the last century several workers supported the hypothesis that occlusion of the vasa nervorum may be responsible for the manifestation of peripheral nervous disorder and for the pathological changes which were occasionally observed in the affected nerves. Among these its strongest advocate was probably Lapinsky, who published in 1899 a detailed review of the subject. More recently clinical evidence in favour of the hypothesis has been collated by Fetterman & Spitler (1940) who, although ignoring the older work on this topic, arrive at much the same conclusion, viz. that vascular disorders may be directly responsible for lesions of peripheral nerves ('ischaemic neuropathies').

The hypothesis, however, though certainly a plausible one, has never been generally accepted, probably because it is largely based on inadequate evidence and unjustifiable inference. For, as a close study of the literature soon shows, there is in most cases no sound evidence that the clinical phenomena are definitely the result of occlusion, either physiological or pathological, of the vascular supply to the nerves concerned; occasionally, it is true, cases have been recorded in which the clinical signs were associated with undoubted pathological changes in the peripheral nerves which in turn presented lesions of their vasa nervorum (Lapinsky, 1899; Woltman & Wilder, 1929; Priestley, 1932; Randall, 1938; and many others). Until, however, it is established that peripheral nerves are normally dependent upon the maintenance of their normal blood supply, and that the interruption of this may result in pronounced physiological effects, it is scarcely justifiable to assert that vascular disorders of nerves may alone be responsible for the various clinical phenomena—flaccid paralysis or disorders of sensibility, or both—which characterize the so-called 'ischaemic neuropathies'.

It is sometimes stated that little or nothing is known about the anatomical and physiological aspects of the vascularization of nerves. This view, however, derives from an ignorance of the literature, since a diligent search reveals a wealth of relevant anatomical and physiological information. It is unfortunate perhaps that the references are so dispersed, uncorrelated and often inaccessible that they have generally escaped attention, and that where isolated references have been used, their full significance has not always been adequately appreciated. As a result, clinical investigation of this problem has taken a somewhat independent course, and in erecting a façade of extremely plausible, but highly empirical, theory, has largely ignored the modern work upon the nature and propagation of the nervous impulse. Since no assessment of clinical findings can be reliable if it does not accord with established anatomical, histological and physiological facts relating to the vascularity of nerves, a review of the literature is clearly desirable, not only to permit an intelligent evaluation of the clinical

contentions, but also to determine the major problems still outstanding in this field of research.

I. ANATOMICAL EVIDENCE

It has long been and still is generally supposed that the normal functioning of peripheral nerves is to a large extent independent of their blood supply, and that the only essential requirement is the anatomical and physiological continuity of the axon with its cell body. That this is, and has always been, the orthodox physiological viewpoint is borne out by the fact that the great majority of physiological experiments involving peripheral nerves entirely disregard their vascularity, on the grounds presumably that nerves are almost avascular structures or at least that their vascular supply is of no great consequence. This, however, is not the opinion of those who have particularly concerned themselves with the vascular anatomy of nerves, and, although their work is generally disregarded, it is surprisingly complete and detailed.

The minute topography of the vasa nervorum has been the subject of several monographs (Hyrtl, 1859, 1864; Quénu & Lejars, 1892, 1894; Tonkow, 1897, 1898; Bartholdy, 1897; and others) which can be supplemented by numerous references to the blood supply of individual nerves or of those of particular regions. But, since it is preferable to elucidate the general principles underlying the vascularization of peripheral nerves rather than to consider in detail the topography and minor variations of the small vessels which supply the various nerves, I shall confine myself to that aspect of the subject.

A. *The peripheral nerves.* While many of the older anatomists were well aware that most nerves received small vessels, Haller (1756) was probably the first to consider them in any detail. Shortly afterwards, Isenflamm & Doerffler (1768), in the first work devoted entirely to the vasa nervorum, discussed at some length their possible significance, while a brief reference to them appeared also in Bichat's text-book (1830). Hyrtl, however, appears to have made the first systematic attack upon the vasa nervorum, and in two papers (1859, 1864) he established that:

(1) Each nerve, large or small, receives nutrient arteries. He described these as very fine vessels which run to the nerve without branching, and after running on its surface enter it and form eventually a capillary plexus which is drained by corresponding veins.

(2) These arteries supply only the nerve, and give no branches to adjacent muscles.

(3) Although concerned primarily with the supply of a certain fixed segment of the nerve, each artery divides into ascending and descending branches, each of which in turn anastomoses with the corresponding branches of adjacent nutrient arteries. There is thus formed within the nerve a continuous longitudinal anastomosis, to which Hyrtl attached great significance in the establishment of a collateral circulation.

The descriptions of the vasa nervorum given by Quénu & Lejars (1890, 1892, 1894) are classical, not on account of their meticulous detail but rather for the clear appreciation of the significance of these minute vessels and in considering them to have an importance beyond that of representing merely potential pathways in the establishment of a collateral circulation. They suggested, for example, that diminished vascularity of the vagus and recurrent laryngeal nerves might account for the difficulties of speech and respiration after thyroidectomy, since in their cervical course these nerves were supplied exclusively by the thyroid arteries. Quénu & Lejars reaffirmed Hyrtl's dictum that all nerves receive a blood supply and emphasized further that:

(1) Each subcutaneous nerve is accompanied by an artery which communicates freely with the general subcutaneous arterial plexus.

(2) A nerve is never supplied by a single artery but receives a number of vessels.

(3) These vessels are constant in their origin.

(4) The nutrient arteries approach the nerve either obliquely or tortuously, and, contrary to Hyrtl's opinion, always divide before entering the nerve into ascending and descending rami, each of which runs some distance superficial to the nerve before entering it.

Shortly afterwards there appeared almost simultaneously Tonkow's preliminary communication on the arteries of the spinal nerves and their ganglia (1897) and Bartholdy's detailed topographical account of the arteries of nerves (1897). Both investigations were carried out quite independently, but produced remarkably parallel results. In 1898 Tonkow published a German translation of his thesis of the previous year. While Bartholdy described the arteriae nervorum in considerably greater detail than Tonkow, concerning himself especially with the manner of their origin from the parent vessels, their direction, and their mode of termination in the nerve, the general conclusions of these two observers show such a close agreement that they may well be considered together. Further, since their generalizations have not undergone any significant change but, in fact, have been largely confirmed by subsequent work, we may regard the summary below as a fair statement of our present knowledge of the anatomy of the nutrient arteries. The more recent work therefore will be referred to only where it supplements or diverges from the conclusions of these two workers:

(1) All nerves, except the terminal ramifications, receive blood vessels which traverse the epineurium to enter the interfascicular connective tissue (perineurium).

(2) Each nerve receives a series of vessels, each of which derives from the nearest available source, i.e. a main arterial trunk alongside which the nerve is running, or, less frequently, one of its muscular branches if this should pass very close to the nerve. Usually, when an artery crosses a nerve which is running by itself it supplies the latter at the point of crossing, except (according to Tonkow) where a distinct fascial plane intervenes (for example, the transverse cervical artery as such never supplies the brachial plexus). It is quite clear, therefore, that the blood supply of nerves is regional.

(3) The origins of the nutrient arteries are not constant, as Quénu & Lejars had maintained, but the variations are related to variations in the arterial system as a whole, and are therefore of no real consequence.

(4) The number of nutrient vessels in any one nerve is not invariable. The limits of variation, however, are what might normally be expected for vessels of their size. Most workers agree that, apart of course from the ganglia, which are well supplied with vessels, the number of nutrient vessels supplying a given length of nerve tends to increase as the nerve passes more distally. Bartholdy attributes this to the fact that as they approach their terminations peripheral nerves tend to enter regions of greater vascularity.

(5) The size of the nutrient arteries varies. Generally it may be said that the larger the nerve the larger the nutrient artery, although Bartholdy prefers to associate together the calibre of the artery and its length. In the larger nerves of adults they may reach a size of $\frac{1}{2}$ mm. (Tonkow) or occasionally 1 mm. (Bartholdy). They are easily distinguishable in late foetuses. Further, when the vessels are smaller they tend to be

more numerous, a fact with which Ramage (1927) is in agreement, but which has been disputed by Petrovits & Szabó (1939), although their figures are not in accord with their text. It may well be, as Bartholdy and Ramage have suggested, that different nerves show quantitative differences in vascularity, and that this may be a factor of some clinical significance. We know very little, however, about comparative vascularity of nerves, or as to whether variations, if present, can be correlated with the greater vulnerability of certain nerves, as suggested by Ramage. Bartholdy states that the same nerve may vary considerably in its blood supply in different individuals.

(6) The length of the nutrient arteries varies. Bartholdy regards length and calibre as being directly proportional to one another. He describes the extraneural course in great detail, emphasizing that, contrary to the opinion of Quénu & Lejars, the nutrient arteries are seldom tortuous, but that they generally run straight or in a very gentle curve from the parent vessels to the nerve. Usually the nutrient artery leaves the main trunk at an acute angle, but occasionally it may be at a right angle or even recurrent. There is a corresponding variation in the approach of the artery to the nerve. He does not agree with Quénu & Lejars that a nutrient artery never enters a nerve perpendicularly, as it is in fact quite a frequent occurrence.

(7) Each nutrient artery, either as it approaches the nerve or after it has entered the epineurium, divides into an ascending and descending ramus. These proceed along the nerve in opposite directions, pursuing courses which are often rather irregular or tortuous, so that they may run axially or reappear from time to time at one or other surface of the nerve. These primary branches of the nutrient artery give off laterally finer arterioles which then branch at right angles and anastomose to form a rectangular arteriolar meshwork; the latter lies in the perineurium, and from it the capillary plexuses are derived. These plexuses alone extend into the endoneurium. The ascending and descending rami of one nutrient artery may be very unequal in calibre. Most nerves usually receive a nutrient vessel at the places where they branch or divide. These vessels generally ascend towards such points and, dividing there, supply one or more ascending rami for the main trunk as well as descending rami for each of its branches. The latter therefore run in a direction the reverse of that of the nutrient artery from which they arise and have consequently been called 'rami recurrentes'.

(8) Adjacent nutrient arteries anastomose with one another. This is achieved primarily by direct communication between the respective ascending and descending rami. Anatomically therefore the nutrient arteries are not end-arteries, and there is, in spite of its fundamentally regional supply, a continuous longitudinal anastomosis within each nerve. The importance of this has long been recognized in connexion with the establishment of a collateral circulation (Holl, 1880; Zuckerkandl, 1885; and many others). That the intraneural arterial anastomosis represents a preformed collateral pathway cannot be denied, and it accounts probably for the continuous haemorrhage which can be readily observed when a 'devascularized' nerve is sectioned peripherally. While agreeing that the longitudinal anastomosis may be capable of enlarging to provide a collateral channel, both Tonkow and Zuckerkandl maintain that it has no special prerogative in this respect over other possible collateral pathways, and that the particular one which develops is determined by a variety of factors. In spite, however, of the importance which has always been attached to the longitudinal anastomosis in this respect, its significance appears to have been considerably overestimated, for it must be emphasized that anatomically the blood supply of nerves is fundamentally

regional and that it is very probable, as we shall see, that each nutrient artery dominates the blood supply of its own particular territory of nerve.

(9) In very large nerves and in the limb plexuses the number of ascending and descending rami may be increased by dichotomous division. These branches may then rejoin and again divide, thus producing within the nerve, or sometimes upon its surface, arterial plexuses—virtual retia mirabilia. The presence of such plexuses has also been confirmed by Petrovits & Szabó (1939). We shall refer to them later in another connexion.

(10) Tonkow has drawn a very careful distinction between:

(*a*) Arteries which serve exclusively or predominantly for the supply of nerves alone. These he regards as true *arteriae nutritiae* (*nutrientes*). Usually each is concerned with supplying a particular section of a nerve, but occasionally they may supply other structures, e.g. an arteria nutritia given off from a large vessel (such as a branch from the brachial artery to the median nerve) may give off small recurrent branches (vasa vasorum) to the arterial trunk and its accompanying vein. The so-called arteria 'comes' nervi ischiadici is in fact an arteria nutritia and not an arteria comes, according to this definition. Again, as Bartholdy and Petrovits & Szabó have also established, arteriae nutritiae may occasionally continue on through a nerve to supply an adjacent muscle (ramus perforans), although Bartholdy points out that such vessels may not always take part in the supply of the nerve through which they pass.

(*b*) Arteries which run in company with nerves, but which are concerned primarily with supplying adjacent tissues, and only secondarily with supplying the nerve which they accompany. These are the *arteriae comites*. The vessels supplying all the cutaneous nerves and a few deeper nerves (median and phrenic nerves) belong here. Tonkow pointed out that both the nerves and the vessels may derive mutual benefit from this intimate association with one another, since the arteries are not only in a position to supply the nerves with their nutrient vessels but are also conveniently situated for receiving from the nerves their own vasomotor innervation. The cutaneous arteriae comites are actually anastomoses between cutaneous arteries supplying the regions through which the nerve is running—they are in fact the 'anastomotic arcades' of Quénu & Lejars. Further, Bartholdy stressed the greater vascularity of the cutaneous nerves, consequent upon their situation in a territory of great vascularity.

We see in the above summary striking evidence against the frequent assertion that little is known of the vascular anatomy of nerves. While it is unnecessary to review in detail the numerous observations which have since appeared, the majority of which fully substantiate the generalizations outlined above, it may be mentioned that the vascular supply of the cranial nerves both inside and outside the cranial cavity corresponds in every respect with that of the spinal nerves (Bartholdy, 1897; Grigorowsky, 1928). The statement by Sänger (1880) that the oculomotor nerve has a 'very rich' blood supply cannot, by itself, be taken to mean that the vascularity of this nerve is any greater than that of other nerves of corresponding size. There is no real evidence that this is so, although some importance has been attached to Sänger's statement in view of the occasional occurrence of intraneural haemorrhage within this nerve (Plaut & Dreyfuss, 1940).

It is well known, of course, that in certain cranial nerves, the vascular supply is somewhat more specialized, a fact which has been regarded by some as having considerable clinical significance (Asherson, 1940). The vascularization of the auditory

nerve, for example, and particularly its cochlear division, has been studied in minute detail by several workers (Konaschko, 1927; Lelli, 1939; and others); while the importance of the arteria centralis retinae, not only to the retina itself but also to the optic nerve (Wolff, 1939), is well known.

Finally, we must briefly consider certain opinions recently expressed by Petrovits & Szabó (1939) which do not agree with the facts outlined above. Their conclusion, for example, that the nutrient arteries are end-arteries is supported neither by their own figures nor by the consensus of informed opinion; while the numerous 'practical' deductions which they derive so speciously from their anatomical observations are entirely unsupported by any evidence, documentary or otherwise. In spite of their claim to 'originality' in methods and results, they have not elicited any new fact of significance.

B. *The spinal and cranial ganglia, and the roots of the spinal nerves.* It is reasonable to suppose that the cell bodies of the receptor neurones in the spinal and cranial nerve ganglia are just as dependent upon their vascular supply as are the nerve cells of the grey matter of the central nervous system, and it might be expected, therefore, that their vascular connexions would have excited a corresponding interest. That this has not been the case is due in part at least to their relatively small size and their inaccessibility.

Adamkiewicz followed up his classical account of the blood supply of the spinal cord with the first detailed description of that of the spinal ganglia (1886), in which he discussed not only the sources of the vascular supply to the ganglia, but also the vascular connexions of the individual ganglion cells, We shall deal here only with the former, and return to the latter in discussing the intraneural plexuses. Adamkiewicz considered that the spinal ganglia received their blood from two sources:

(1) A direct supply from the corresponding segmental artery by way of its spinal branch. In the case of the cervical ganglia where the spinal vessels arise from the vertebral artery, the latter also contribute direct branches to the ganglia. Generally, however, it is the radicular branch of the spinal vessels which is the primary source of supply. These are the most important sources, and the only ones where the complementary supply is absent (thoracic region particularly). Adamkiewicz (1882) also described the anterior radicular arteries as varying in number between three and ten, while the posterior, although more numerous (16–17), were said to be generally smaller, and in the upper cervical region usually completely absent. In the regions of the plexuses the posterior radicular arteries were said to be larger, and the brachial plexus in particular seemed to have a preferential blood supply in that each root was invariably accompanied by a radicular artery.

(2) An indirect supply, by vessels passing outwards in the nerve roots (particularly the dorsal nerve root) from the vascular channels on the surface of the spinal cord (anterior and posterior spinal arteries).

Bartholdy (1897) and Tonkow (1898) later showed that the ganglia conform to the accepted rule in obtaining their blood supply from the nearest available sources. These are generally several, and at the very least two (Tonkow). The ganglia receive vessels at both their anterior and posterior surfaces. The larger ones (those of the plexuses) receive several nutrient arteries, each of which may branch before entering the ganglion, so that as many as six or seven vessels may ultimately reach the ganglion. Anserow's results (1922) agree essentially with the above.

Recently, however, Bergmann & Alexander (1941) have studied the blood supply of the spinal ganglia in much greater detail. They agree that the principal supply of the ganglion is from the spinal branch of the corresponding segmental artery, but state that both the anterior and posterior radicular branches of the spinal artery are involved. They point out, significantly, that the radicular arteries devote themselves almost entirely to supplying the ganglion, and though they often continue on to the spinal cord along the anterior and posterior roots (which they also supply), they are then considerably reduced in calibre. The spinal artery itself also supplies the commencement of the spinal nerve up to and including the commencement of its anterior and posterior divisions. There are two types of ganglionic arteries which anastomose within the ganglion: (*a*) those which, after piercing the dural sheath, break up into arterioles which supply the periphery of the ganglion; and (*b*) others which enter the ganglion at its extremities. Of the latter those branches which enter medially are frequently tortuous at first, but finally run axially to feed the plexuses in the central part of the ganglion; these vessels anastomose with the arterioles and capillaries of the posterior root which are also derived from the posterior radicular artery. Few vessels enter the lateral pole of the ganglion which is supplied mainly by a continuation of the intrinsic plexus. There is only a feeble anastomosis here with the capillary plexus of the spinal nerve, so that the longitudinal arterial pathway of the latter does not have significant connexions proximal to the commencement of the nerve.

The ganglia of the cranial nerves have never been specially investigated. In the systematic accounts of the blood supply of the cranial nerves (Bartholdy, 1897; Grigorowsky, 1928) only the macroscopic anatomy of the vessels passing to the various ganglia is discussed. The ganglia of the vagus, for example, appear to be supplied principally by three or four vessels from the ascending pharyngeal artery or its posterior meningeal branch, as well as by branches of the superior laryngeal artery (which Quénu & Lejars regarded as the most important supply). The trigeminal ganglion, whose blood supply may have considerable significance, has, according to Bartholdy and Grigorowsky, several sources of supply: (*a*) the internal carotid artery, which provides branches (arteriae sinus cavernosi) of moderate size to the medial side of the ganglion. These vessels also are entirely responsible for the supply of the first division of the trigeminal nerve, and largely so for the second, in their intracranial course; (*b*) the middle meningeal artery, branches of which reach the outer side of the ganglion; and, finally, (*c*) the arteria meningea parva, which passes to the ganglion through the foramen ovale. The interest of the vascular supply of this ganglion lies in its possible significance in the aetiology of trigeminal neuralgia (Adams & Robinson, 1941), while its differential blood supply may be correlated with the well-known tendency of the ophthalmic division to escape in this disorder. It should be pointed out, however, that others do not agree that the meningeal arteries take part in the vascularization of the trigeminal ganglion. Tanasesco (1905) states emphatically that the ganglion derives its supply from the internal carotid artery, and that the ganglionic branches do not anastomose with the middle meningeal artery or the meningea parva; while Ferrari & Ribet (1927) and Stöhr (1928) mention only the internal carotid, and ignore entirely the meningeal arteries.

C. *The sympathetic nervous system.* While the blood supply of various parts of the sympathetic nervous system is not infrequently referred to in the older literature, the first systematic descriptions appear in the several papers of Delamare, Tanasesco and

Le Sourd, during 1905 and 1906. Bartholdy had previously discussed very briefly the arteries supplying the sympathetic chain, and more recently Latarjet & Bonnet (1913) described that of the hypogastric plexus, while Anserow (1922) reinvestigated the blood supply of the sympathetic chain. The latest comprehensive account, however, is that of Machado de Souza (1932), which forms the basis of the following summary.

Each ganglion of the paravertebral chain receives several vessels from neighbouring arterial trunks. The number of vessels going to any one ganglion is variable (one to seven) and does not correspond to the size of the ganglion (Anserow). The ganglionic vessels generally arise from the corresponding segmental artery (intercostal or lumbar), with modifications in the cervical and sacral regions. The nutrient vessels usually divide before reaching the ganglia and then supply branches to the interior, which form there an intraganglionic plexus much as in the spinal ganglia; some branches, however, do not enter the ganglion, but ramify on its surface, and by anastomosing freely with neighbouring branches form there a periganglionic plexus, which is prolonged on to the interganglionic trunks. Some branches may enter the ganglion, and traverse it to reappear on the opposite surface. It will be seen, therefore, that there is a general similarity between the sympathetic and spinal ganglia in respect of their vascular supply.

The rami communicantes vary in their mode of supply; the longer rami receive special vessels, while the shorter ones are supplied by a prolongation into them of the intraganglionic plexus. The vessels of the rami, furthermore, anastomose freely with those of the spinal nerves with which they are in relation, and are not independent of the latter as some have contended.

The splanchnic nerves, proximally, receive a continuation of the superficial ganglionic plexus, although this is soon reinforced by successive branches of the intercostal arteries. On approaching the nerves these vessels behave like those of the peripheral nerves, i.e. they divide into ascending and descending rami which form a continuous anastomosis along the nerves as far as the previsceral ganglionated plexuses. The latter are supplied by vessels in their vicinity, i.e. either from the aorta itself, or, more frequently, certain of its branches. Not only, therefore, is there a close correspondence in their mode of supply between the sympathetic and the peripheral nerves, but there is also a continuity between the vessels of the two by way of the rami communicantes.

D. *The intraneural plexuses.* The intrinsic vascular pattern of the nerve trunks and of the various ganglia has been frequently studied by a variety of methods, none of which has been entirely successful owing to the difficulty of ensuring satisfactory visualization of the normal vascular bed. The following methods have been employed with varying success:

(1) Direct observation. This has been extensively developed by Vonwiller (1927), who employed incident in place of transmitted illumination. He has recently applied it to microscopic investigation of peripheral nerves in the living animal (1933, 1934), and records that by this method Wosskressensky managed to observe the circulation quite clearly on the surface and within the rami communicantes of the living frog. Such a method, if further developed, may prove to be of extreme value by making it possible to study the reactions of the vascular bed of a nerve in vivo.

(2) Injection. The value of conventional procedures in the study of the finer vascular ramifications has been vigorously contested. They have been often employed, however, and were used by Adamkiewicz (1886) for the spinal ganglia, and by Dunning

& Wolff (1937) for the trigeminal ganglion, and in both cases the injected tissues were sectioned for histological study. In other cases, however, injected nerves and whole ganglia were cleared to retain as far as possible the three-dimensional pattern (Buitink, 1934; Machado de Souza, 1932; and others).

(3) The benzidine technique, which has been claimed to possess certain advantages over the injection methods, in retaining the normal number and calibre of the intraneural vessels, has been successfully employed by Bergmann & Alexander (1941) in their study of the intrinsic vascularity of the spinal ganglia. It is technically a much more difficult procedure in the case of the ganglia than it is with tissues of the central nervous system, so that criticisms of this technique apply here with even greater force.

(4) Neurography. The filling of the vascular bed of the peripheral nerves by opaque injection with subsequent visualization by X-rays has been long used in the study of the intraneural plexuses. While it holds certain minor advantages over ordinary injection, it has not in fact proved much more valuable. Nevertheless, it has been extensively employed by many workers including Chaumet, Heymann & Mouchet (1921), Ramage (1927), Monteiro, Rodrigues *et al.* (1935), Picco (1936), and Petrovits & Szabó (1939).

By one or other of these methods it has been conclusively demonstrated that every nerve contains a comparatively rich vascular plexus, the meshes of which are arranged longitudinally. The division of the nutrient arteries into ascending and descending rami and their anastomosis to produce the continuous longitudinal pathway along the nerve have been abundantly confirmed. Buitink (1934) states that accessory longitudinal anastomoses may also be seen connected to the main one, in a ladder-like manner, by transverse anastomoses. Petrovits & Szabó (1939) figure similar and even more complicated arrangements.

The ganglia are much more vascular than the nerves, as indeed we might expect from their respective metabolic requirements, but precise descriptions of their vascularity are few. Adamkiewicz (1886), in one of the earliest descriptions, gives a completely iconoclastic account of the circulation through the ganglion, which has been severely criticized by Stöhr (1928). Adamkiewicz considered that the ganglion cells and their capsules lie in the relatively wide meshes of an 'arterial' and a 'venous' plexus; that proceeding from the 'arterial' plexus, and connected to it by minute afferent and efferent vessels, are dilatations which actually occupy the subcapsular space and invest the cell bodies like a chalice, bathing them directly with plasma, since his afferent and efferent vessels were believed to be too small to allow the passage of the corpuscles. While it seems obvious that Adamkiewicz was merely describing injection artefacts, it is remarkable that Bergmann & Alexander have also described curious dilatations of the capillaries of the spinal ganglia. They have shown that there is a very dense capillary plexus within each ganglion, which they regard as comparable with that of the grey matter of the central nervous system, although Dunning & Wolff (1937) had previously contended that the vascularity of the ganglia is not as rich as that of the grey matter. The capillaries and precapillaries of the ganglion show 'bead-like dilatations', which are simply localized enlargements of the capillaries, normally reaching to three times their average calibre, although in older people they may show a further considerable increase in size. They seem to be peculiar to man. Although it is quite obvious that the dilatations described by Bergmann & Alexander do not possess the very intimate relationship to the cell body which Adamkiewicz claimed for his, it

may well be, since they correspond roughly in size with that of the ganglion cell, that the latter was actually observing similar structures. Bergmann & Alexander correlate the increase in size of the dilatations with the recorded decrease of ganglion cells with age. They could not decide, however, whether the dilatations represent local 'aneurysms' which cause the degeneration of adjacent cells, and thus account for the gradual reduction in sensibility which has been described in older people, or whether the atrophy of the cell is primary, and precedes the formation of the dilatation which may then replace it. In the latter connexion they suggest that the tortuosity of the intraganglionic vessels at their commencement is an 'inefficient mechanical arrangement' which might well predispose to cell atrophy—although Quénu & Lejars (1892) held precisely the opposite view in regarding the tortuosity of nutrient vessels as a protective mechanism.

In a description of the age changes in peripheral nerves, Cottrell (1940) emphasizes the important age changes in the intraneural vessels with which, although not accompanied by dramatic changes in the nervous tissue itself, she also associates the recorded clinical evidence of vague motor and sensory changes in old age.

There is therefore in every nerve an extensive intraneural plexus, whose meshes extend into the endoneurium in relationship with the nerve fibres. In the various ganglia the capillary plexus is much denser, and closely enmeshes the cell bodies of the neurones. In the spinal ganglia the capillaries present curious dilatations. In addition to the fundamental plexuses, there is in each nerve trunk a distinct macroscopic longitudinal arterial pathway, formed by primary anastomoses between the ascending and descending rami of successive nutrient arteries. Finally, age changes have been shown to occur in the vessels both of the ganglia and of the nerve trunks, which have been associated with changes in the nervous tissue itself, although the precise relationship between such changes is not yet clear.

E. *Embryology.* Certain large, so-called 'anomalous', vessels which in man occasionally accompany certain nerves (median, sciatic, saphenous, lateral popliteal, etc.), and which may be normally present in other mammals, have always excited considerable interest. Have they any bearing on our problem?

Tonkow (1907), in an inquiry into the embryological significance of the neural vessels and the development of these 'anomalies', emphasizes the primitive relationship between the developing nerves and arteries of the extremities. On the evidence of de Vriese (1902) and Göppert (1905), who have shown that primitively each nerve is intimately related to a developing vascular plexus, he points out that a nerve which, in the adult, appears to be running an independent course, does in fact still retain its primitive vascular relations, either in the form of an arteria comes or, even less obviously, as the longitudinal anastomosis, fed by the successive nutrient arteries of the nerve. The 'abnormal' retia mirabilia, which attracted Müller's attention (1903) and which he appeared to regard as something unique, Tonkow regards as quite a normal feature of any of the larger nerves, and this has since been confirmed by Petrovits & Szabó (1939). Again, certain nutrient arteries are most likely only vestigial portions of definite embryological arterial pathways; for example, the first and most constant nutrient artery of the median nerve ('Arteriola fundae nervi mediani' of Robinson, 1910), which it receives from the axillary artery at the junction of its two roots, seems to be the proximal portion of the primitive superficial brachial pathway, since it appears as a branch of the superficial brachial artery when this is present definitively. So, also,

the arteria 'comes' nervi ischiadici represents the persistent proximal portion of the primitive arteria ischiadica (which is a true arteria comes if it is present definitively), now restricted entirely to the supply of a portion of the nerve and therefore, by definition, a true arteria nutritia.

The interest which has always been taken in such 'abnormal' vessels when they appear accounts for the undue emphasis which has attached to certain arteriae nutritiae and arteriae comites, whereas, of course, they have no special significance at all as far as the nerve is concerned, since they resemble in every respect arteriae nutritiae and arteriae comites elsewhere. Since, then, their functional significance must correspond to that of similar vessels, any further consideration of their phylogenetic and ontogenetic history can be of no assistance to us.

F. *Innervation.* It is well known that the larger branches of the nutrient arteries running in the epineurium and perineurium have a well-developed tunica media, composed of smooth muscle fibres; it is a just inference therefore that these vessels are contractile. Furthermore, non-medullated nerve fibres (nervi nervorum) have been demonstrated in the epineurium and perineurium. Certain of these have been shown to terminate in end-bulbs and have therefore been regarded as sensory in function (Horsley, 1884; Prus, 1886). Others, however, are intimately associated with the intraneural vessels and are probably vasomotor in function, i.e. they are postganglionic sympathetic excitor fibres supplying the arteries of the nerves (Sappey, 1889). It is clear, however, that our information on this subject is most inadequate. We have no precise information as to the significance to the vasa nervorum of their innervation, and it has even been suggested (Bülbring & Burn, 1939) that the vasa nervorum may resemble, in their reactions, the cerebral vessels—a view which recalls a similar opinion strongly held by Bichat and by Quénu & Lejars. In the case of the optic nerve it is of interest to note that the arteria and vena centralis retinae are also accompanied by a plexus of non-medullated (? sympathetic) fibres, the so-called 'nerve' of Tiedemann.

In spite of our ignorance of the significance of the vasa nervorum, we find that clinical practice has far outstripped factual knowledge, and that certain manœuvres—cervical sympathectomy, alcoholization of the adventitial plexuses of the common, internal and external carotid arteries—have been practised on the very empirical assumption that the vasoconstrictor impulses to the vessels of the trigeminal nerve were thereby interrupted, and that a dilatation of these vessels resulted (Kulenkampff, 1926; Wischnewsky, 1926; Nasaroff, 1927). Although they may ultimately prove to have been justified, there is at present no scientific basis for such procedures.

G. *The veins of nerves.* So far we have considered only the nutrient arteries of nerves and their distribution, mainly because these alone have been investigated by the majority of workers studying the vessels of peripheral nerves. Indeed, only Quénu & Lejars seem to have made any systematic attack upon the venous drainage of nerves, which is perhaps unfortunate in view of the likelihood that disturbances on the venous side may have an importance as great as that claimed for arterial lesions. This has, in fact, been mentioned clinically in connexion with varices of the venae 'comites' nervi ischiadici and associated phlebogenic neuropathy (Quénu, 1888; Rheinhardt, 1913; Edinger, 1914).

Bichat (1830), in a brief account of the veins, claimed that they resemble the arteries in their arrangement within the nerve, in forming similar longitudinal anasto-

motic plexuses, but that, in the larger nerves at least, they resemble the veins of the cerebrum in leaving the nerves independently of the incoming nutrient arteries.

Quénu & Lejars (1892, 1894) elaborated the following principles:

(1) The veins of the cutaneous nerves, curiously, do not enter the large cutaneous veins in their vicinity, but drain deeply, through the deep fascia, into the underlying muscular veins. Connexions between the veins of the subcutaneous nerves and adjacent veins, although present, are quite insignificant.

(2) The veins of the deep nerves drain directly into muscular veins, or, when the nerves are in a neurovascular bundle, they may also drain into the venous plexus of the arterial wall (vasa vasorum), crossing over the main vein, or the venae comites of the artery, to do so. Only rarely do they open directly into the latter.

Quénu & Lejars regard such arrangements as indicating a preferential venous drainage for peripheral nerves, since they assume that muscular activity, and possibly arterial pulsation as well, ensure the rapid and efficient removal of blood from the nerves.

(3) The mode of emergence of the veins from the nerves and their intraneural pattern correspond essentially to the arterial arrangement. The veins do not generally emerge from the nerve independently (as Bichat believed) but accompany the nutrient arteries. The tendency of veins within the nerve to be rather more tortuous than the arteries they regard as a predisposing factor in the formation of intraneural varices.

The brief description given by Rheinhardt (1913) of the veins of the sciatic nerve adds nothing to the above. The veins of the spinal ganglia, however, were not described by Quénu & Lejars. They appear first to have been described by Adamkiewicz (1886). His insistence, however, upon an intracellular connexion of his 'venous' plexus with the interior of the ganglion cell is not supported by the recent work of Bergmann & Alexander, who state that the veins drain from the capillary bed of the ganglion mainly towards its periphery where they open, together with the veins of the roots, into a rich extradural plexus. About the extremities of the ganglia are 'vascular collars' into which drain the veins emerging there. The extradural plexus communicates freely with the extradural veins of the vertebral canal (internal vertebral plexus) as well as with the external vertebral plexus, the intervertebral veins and the segmental veins. They contend that because of this free communication with the vertebral plexuses, the venous drainage of the ganglia has largely lost its segmental character.

II. EXPERIMENTAL EVIDENCE

The anatomical evidence clearly demonstrates that all nerves receive a relatively substantial blood supply; that the nutrient arteries are several in number for each nerve and are derived locally, either from adjacent major arteries or from accompanying arteriae comites, so that *the blood supply of nerves is fundamentally regional*; that, since these regional arteries anastomose within the nerve, there is consequently superimposed upon the basic regional supply an extensive longitudinal arterial pathway accompanying the nerve throughout its length. There is therefore a close similarity between the vascular arrangements of a peripheral nerve and those of the spinal cord, although it must be stressed that the nutrient vessels of a nerve cannot be regarded, on anatomical grounds at least, as simply 'reinforcing' vessels for what has often been called the 'proper' artery of the nerve, i.e. the longitudinal vascular pathway. Such a

view would imply not only the dominance of the latter over the regional supply but also a corollary: that devascularization of a nerve along its length, by removing the regional sources of supply, would in no way impair its nutrition. The physiological relationship of the regional and the longitudinal sources in the nutrition of the nerve becomes therefore a problem next only in importance to that of determining the part which is played by the vascularity of peripheral nerves in their normal functioning.

As has been pointed out, it has long been customary either to ignore completely the vascularity of the nerves or to regard it as a factor of no real consequence. There have therefore been few attempts to elucidate the functional significance of the vasa nervorum, and few authorities who have given serious consideration to the possibility that its vascularity may play a responsible part in maintaining the integrity of a normally functioning nerve. Hence, since there has been no co-ordinated attack upon this problem physiologically, and since many aspects have been only superficially investigated, a review of the relevant evidence must necessarily appear somewhat disjointed, and the deductions drawn can only be of a very general nature.

A. *Direct evidence.* This, so far, has been derived entirely from animal experiments. It is important, therefore, to note that, except for minor anatomical detail, the general arrangements of the vasa nervorum are virtually the same in all mammals (Tonkow, 1898); in lower vertebrates, however, there are considerable differences (Mies, 1926). Thus we may justifiably assume that the objective results obtained experimentally in lower mammals are applicable to man. Such is of course in accordance with accepted physiological procedure, and is justified where technical or other difficulties militate against human experiment.

The earliest available account of investigation of a nerve after devascularization is that of Mayer (1878). He found that the devascularized facial nerve in situ in the rabbit lost its excitability in 15–30 min. A corresponding experiment was carried out on the sciatic nerve of the rabbit by Fröhlich & Tait (1904) with a similar result. However, in view of the difficulty of complete devascularization of a peripheral nerve (Koch, 1926) and also of avoiding trauma to the nerve in the process, the above experiments require confirmation.

In 1905 Okada produced degeneration of the sciatic nerve of the rabbit merely by ligating the inferior gluteal artery. This important observation has never been confirmed, but if correct it is extremely significant because it implies, first, that occlusion of a single regional vessel exerts a profound effect upon the nerve which it supplies, and secondly, that here is a type of degeneration which differs fundamentally from typical Wallerian degeneration in its manner of production, even if the effects are similar. Wallerian degeneration connotes essentially a structural interruption in the continuity of the axis cylinder. Here, however, no such interruption has occurred—complete anatomical and physiological continuity between the cell body and the whole length of the axis cylinder still persists. If therefore this isolated result should be substantiated it is clear that we must differentiate an *ischaemic* from a *Wallerian* degeneration of peripheral nerves. The implications of this experiment are so far-reaching that it demands immediate verification.

More recently Koch (1926), also using the sciatic nerve of the rabbit, has studied the effects of ischaemia upon the injury current in this nerve. In spite of considerable difficulties, principally relating to the elimination of collateral pathways, he established the following facts:

(1) In a nerve whose vascular connexions are intact the current of injury is smaller and disappears more rapidly than in an isolated nerve.

(2) In a vascularized nerve the onset of ischaemia (by occlusion of the aorta, all possible collateral pathways having been ligated) is accompanied by an immediate ('anaemic') increase of a subsiding injury current. This in turn proceeds to subside as the ischaemia is maintained, but as soon as the circulation is released there is recorded a further ('metanaemic') increase. Koch was unable to explain the significance of these effects, but it is clear that not only ischaemia itself but also revascularization after a period of ischaemia both produce pronounced, if possibly different, physiological changes within the nerve; changes which were shown by Koch to be almost certainly due to *local* vascular changes within the nerve.

(3) Koch also investigated the extent to which the different regional vessels supplying the sciatic in the thigh contribute to the total vascularity of the nerve, i.e. he attempted to define their physiological zone of supply. The results he obtained are convincingly in favour of the dominance, in this nerve at least, of the regional supply over the longitudinal, although the latter may compensate to a slight extent for the elimination of a regional vessel. He points out here that there is some regional variation in the vascularization of the rabbit's sciatic nerve—the distal part of the nerve, for example, appears to have a rather better blood supply than the proximal.

The results obtained by Gerard (1930), although dealing with isolated nerve, are pertinent to our discussion, since they indicate that significant differences are observed in the resting and action potentials before and after asphyxia. Asphyxia produces pronounced changes in the action potentials which culminate in nerve block, although all the fibres do not block simultaneously but probably according to their diameter. Furthermore, he shows that mammalian nerve (dog) asphyxiates more rapidly than submammalian (frog), which suggests a higher metabolism in mammalian nerve and a greater dependence of the nerve on its blood supply. Finally, he demonstrates that exhibition of oxygen to an asphyxiated nerve not only restores the action potentials but that these are at first greatly exaggerated.

The recent results of Bülbring & Burn (1939) also appear to indicate that the functioning of peripheral nerves is influenced by their vascular supply. They found that continued stimulation of the anterior roots concerned in the supply of the gastrocnemius muscle resulted in a progressive reduction in the response of the muscle until finally it ceased to respond. Investigation showed clearly that the fault here lay neither in the muscle nor the neuromuscular junction but actually in the sciatic nerve itself. A nerve block had, in fact, resulted as 'the vascular tone became reduced'. A significant outcome of the experiment was the fact that whilst other experiments had led 'to the assumption that the chemical requirements of nerve fibres are so small that they would be satisfied so long as some circulation is maintained no matter how small this circulation may be, our evidence indicates that this is not so and that excitability or conductivity can fail while a blood flow still persists'. Ischaemia therefore need not necessarily be complete in order to produce significant effects on the transmission of the nerve impulse.

They found it more difficult to explain, however, how the impulse came to be restored either by the addition of vasoconstrictor substances (adrenalin, pituitrin) to the perfused blood, or by stimulation of the lumbar sympathetic chain. This curious result seems paradoxical since, if the vasa nervorum respond, as we might expect, in

the same way as muscular vessels generally, the effect of such procedures should obviously be to reduce still further the vascular supply to the nerve. On the other hand the suggestion that the vasa nervorum may resemble the cerebral vessels in their passive response to an increase in the general systemic pressure, although harmonizing with the views of Bichat and Quénu & Lejars, seems to have little to commend it. Bülbring & Whitteridge (1941) have since reinvestigated the influence of adrenalin on nerve fibres, and find that its primary effect is not upon the vasa nervorum but directly upon the nerve fibres themselves in which it induces physiological changes. Nevertheless, it is clear that the responses of the vasa nervorum to various stimuli, nervous and pharmacological, require considerable investigation.

Parrack (1940) calls attention to another significant difference between the physiological state of vascularized nerve and that of isolated nerve or a nerve whose blood vessels have been ligated, since in vascularized nerve there is no progressive decay of the electrotonic state such as occurs in isolated nerve, which also accords with comparable results previously obtained by Liesse (1938 *a*, *b*). As these investigations were carried out on the nerves of a cold-blooded vertebrate (frog), such results, as suggested by the experiments of Koch and Gerard, should be even more striking in the case of mammalian nerve.

B. *Indirect evidence.* The difficulty of direct interference with the blood supply of nerves in man has been overcome in an ingenious manner by Lewis, Pickering & Rothschild (1931). Using a special clamp they claim to be able to render ischaemic a section of a nerve trunk, without affecting the circulation in the limb, and therefore presumably in the nerve also, distal to the clamp. In this way they ensure that the effects observed by them can be attributed entirely to ischaemia of the section of the nerve beneath the clamp. The criticism of Bishop, Heinbecker & O'Leary (1933), that the effects observed were due not to ischaemia of the nerve trunks but to direct pressure upon them, although it appears to have received little support, cannot be entirely ignored, since, if the longitudinal anastomosis within the nerve has the significance usually attributed to it, it would appear to be impossible to interrupt completely the blood supply to a nerve of any size without at the same time producing direct pressure upon the nerve fibres. If, however, the *regional* blood supply to the nerve is predominant, as most of the anatomical and physiological evidence leads us to suspect, then the production of a local ischaemia without contemporaneous pressure effects becomes a possibility. So, in a way, we may regard Lewis's experiments as affording some measure of inferential support to the latter conclusion. A further point in relation to this is the possibility, to which Lewis *et al.* refer on p. 25, that, in the majority of cases the ischaemia of the nerves may not have been complete, which they account for by 'a certain amount of diffusion... between the nerve and the surrounding tissues to which the blood supply is maintained'. This rather vague statement may have a more precise explanation, on anatomical grounds, in the slight compensatory influence of the intraneural longitudinal anastomosis, particularly since Bülbring and Burn have shown that the production of fibre block does not necessarily imply total ischaemia, and that it occurs in a vascularized nerve whose circulation is diminished below a certain level (not defined). Lewis *et al.* suggest, however, that when the compression involves a nerve which lies in direct relationship with a bony surface the ischaemia may then be complete, since in that case the effects of the compression occur simultaneously and uniformly over the whole area (distal to the clamp) supplied by

the nerve as compared with the centripetal progression of the effects in the case of other nerves.

We may therefore accept the following results as being most probably the physiological effects of ischaemia (partial or complete) of a localized portion of a nerve trunk. The effects comprise widespread disturbances of sensibility and of motor activity, embracing the whole area of distribution of the nerve distal to the ischaemic zone:

(1) There is a progressive loss of all forms of sensibility. This sensory loss, which was subsequently reinvestigated by Lewis & Pochin (1937), occurs after an initial delay of 15–20 min., which is increased as the clamp is placed more distally. There is an almost simultaneous loss of touch, temperature sensibility, and fast-conducted (first) pain, while the defect in slow-conducted (second) pain occurs slightly later. The loss of all forms of sensation commences distally and then spreads centripetally until eventually the entire area distal to the clamp supplied by the nerve becomes completely anaesthetic. Owing to slight variations in the rate of disappearance of the different forms of sensibility, some dissociation between them, and particularly between first and second pains, was observed. This differential loss of the various forms of sensation probably has some relation to fibre size, but the correlation is not absolute (Lewis & Pochin, 1937–38, p. 149).

(2) The motor functions are likewise affected in a centripetal direction, and all the muscles supplied by the nerve (distal to the clamp) eventually fail to respond to nerve stimulation. The pilomotor nerves, which are also ultimately involved, retain their function much longer than those which supply skeletal muscle.

The results of other experiments involving complete occlusion of the circulation through a limb so closely parallel those obtained by using the special clamp as to justify the assumption that here also the nervous effects are probably due to ischaemia of the nerve trunks. This view has received the support of Gasser and his collaborators (Gasser, 1934; Clark, Hughes & Gasser, 1935) and of Thompson & Kimball (1936).

The evidence that such profound effects may result simply from a localized ischaemia of a nerve trunk is of course of the utmost significance, both physiologically and clinically. There appears, indeed, to be a correlation between the results just described and those of Koch and Gerard. Gerard showed that nerve block results from asphyxia and that exaggerated action potentials follow the restoration of oxygen, while Koch also found that two responses were obtained in the nerves he investigated, the first accompanying the onset of ischaemia, and the second occurring on revascularization. Lewis *et al.*, on the other hand, observed that ischaemia of a localized portion of a nerve results in the 'ischaemic paralysis' described above, and further, that revascularization, as well as abolishing the paralytic effects, produces changes within the nerve which are interpreted as the subjective phenomenon of tingling.

The conclusions derived from such observations on normal subjects lend considerable support to the hypothesis advanced by Lapinsky (1899) that in sudden occlusion of a main limb vessel, as may occur by thrombosis, embolism or spasm, the accompanying motor and sensory effects are primarily the result of ischaemia of the nerve trunks, although he did not agree that similar effects followed gradual occlusion of the vasa nervorum as many others have maintained. Lapinsky demonstrated in some of his cases definite degeneration of the nerve fibres, while in others where a collateral circulation was rapidly established there was a consequent restoration of function of the nerves. Under such circumstances therefore the ischaemic change in

the nerves is apparently reversible, which is also supported by the observation of Lewis *et al.* that revascularization of a previously ischaemic nerve results in a rapid recovery of all forms of sensation, and in an order the reverse of that of involvement. They pointed out also that the subjective tingling which is associated with revascularization is all the greater the longer the ischaemia has been in force. There is every reason to believe that this phenomenon also is due to changes occurring in the nerve trunk at the actual site of the previous ischaemia.

In support of the evidence outlined above, Wortis, Stein & Jolliffe (1942) present clinical and experimental data, the significance of which is also fully discussed.

SUMMARY

Considerable anatomical evidence shows beyond doubt that all nerves receive a blood supply, and that it is derived from regional vessels which contribute, however, to the formation of a longitudinal vascular pathway along the nerve. The various ganglia associated with peripheral nerves receive a much richer blood supply than that of the nerves themselves, although derived similarly from vessels in their vicinity.

Such experimental evidence as is available indicates that its vascularity is of importance to a nerve, and that cessation of the blood supply to any part of a nerve affects the passage of the nerve impulse and will ultimately induce a complete nerve block. The larger, rapidly conducting, fibres appear to be the first affected, and the ischaemic paralysis is completed by the involvement of the slow-conducting fibres. These changes are reversible, and restoration of the circulation through the nerve not only restores its capacity to conduct but initiates exaggerated responses, which may be interpreted as the subjective phenomenon of tingling. There is as yet, however, no sound evidence that other subjective symptoms, such as pain and vertigo, may also result from ischaemia of nerve. The view that ischaemia per se may induce changes at the site of its occurrence which may be interpreted as pain has frequently been put forward (Leriche, 1939). While such a view has never received much support it is not impossible that the occurrence of pain may be correlated with the fact that ischaemia involves the rapidly conducting fibres first. According to the views of Pochin (1938) and Gasser, if the impulses travelling along the slowly conducting (*C*) fibres are released from the 'damping' effect of those passing in the rapidly conducting fibres, pain of a severe type results. So that stimuli which would not normally result in pain might become effective when the propagation of the impulse along the rapidly conducting fibres is abolished by ischaemia.

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AGE AND SEX VARIATIONS IN THE FAT OF THE ADRENAL CORTEX OF THE WHITE RAT

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The general distribution of fat in the adrenal cortex of the rat is described by Jackson (1919) and Watson (1927), but no detailed study seems to have been reported. The present paper is a parallel to the systematic studies of Whitehead on the mouse (1933*a*, 1934*a*), guinea-pig (1934*b*, 1935*a*) and rabbit (1936). The results form a basis for experimental work on cortical fat and also bear on the problem of cortical structure.

MATERIAL AND METHODS

The adrenals of 120 normal albino rats of the Wistar strain were studied. The rats were killed so as to form six age groups each consisting of ten males and ten females. The body weights just before death are shown in Table 1. The dates of birth of all rats not over 169 days old were known; none of these rats had been used for breeding. The rats forming the group 'over 169 days' old were probably over a year old; these had been used for breeding and were killed when considered too old to be used again

Table 1. *Body weights (g.) of normal rats*

Age days	Males		Females	
	Mean	Range	Mean	Range
<1	5·7	5·0–6·4	5·0	4·4–5·5
14	23·0	21·0–24·0	22·6	21·0–25·0
29	64·6	58·0–77·0	64·3	53·0–71·0
84	222·0	186·0–274·0	163·8	146·0–180·0
169	288·4	234·0–340·0	189·1	168·0–226·0
>169	293·0	242·0–340·0	225·8	217·0–238·0

Each age group consisted of ten males and ten females: total 120.

for this purpose. The diet consisted of Purina fox chow meal and water. Vaginal smears were made on the thirty sexually mature females for 4 days before death, the technique of smearing and method of interpretation being those of Long & Evans (1922). Four rats were killed in pro-oestrus, seven in oestrus, three in metoestrus and fourteen in dioestrus; the phase of the other two was uncertain. Four rats were killed by a blow on the head, all the others by decapitation.

Both adrenals of each rat were fixed for at least 2 days in 10 % formol saline, prepared by dissolving 8·5 g. sodium chloride in 100 c.c. commercial formalin and diluting the solution to 1 l. with distilled water. Transverse sections cut at 25 μ on a freezing microtome were (*a*) stained for fat with Sudan IV without counterstain, and (*b*) treated by the Schultz method for cholesterol (Kay & Whitehead, 1937).

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RESULTS

The appearance of cortical fat was closely similar in both adrenals of any rat. The range of variation was the same in both sexes, but there were sex differences in the frequency of certain appearances. There was no evidence of any relation between fat and the oestrous cycle. The Schultz test showed that all the fat in all the adrenals contained cholesterol, the distribution of cholesterol being the same as that of fat.

According to its fat content the cortex was divisible into four concentric zones (Pl. 1, fig. 1): (1) a narrow subcapsular zone which was always fat-laden, (2) a narrow zone in which fat was rarely obvious, (3) a broad zone nearly always fat-laden, and (4) a broad juxta-medullary zone with a widely variable fat content. Fat-containing cortical cells were also seen in the medulla of all the adrenals of the newborn rats (Pl. 1, fig. 2) and of one or both adrenals of some rats of each sex at later ages.

The only important variations were in zones 3 and 4. Zone 3 showed regular age variations in size; since the size of zone 3 varied inversely as the size of zone 4 it is unnecessary to give results for both zones, and it will be more convenient to give them for zone 4. Zone 3 showed no obvious regular variation in fat content, but there was one conspicuous irregular variation, namely, the almost complete absence of fat from zone 3 in both adrenals of each of the following rats: at 14 days, a 24 g. male and a 22 g. female; at 29 days, a 61 g. male; at 84 days, a 156 g. female. Zone 4 showed regular age variations in fat content as well as size, and these will now be described in detail.

Table 2. *Numbers of rats showing various sizes of zone 4 at various ages (days)*

Breadth of zone 4 / Breadth of cortex	Males						Females					
	<1	14	29	84	169	>169	<1	14	29	84	169	>169
2/3*	—	6	1	—	—	—	—	7	—	—	—	—
1/2	—	4	7	6	2	1	—	3	—	3	—	—
1/3	—	—	2	3	8	7	—	—	9	7	2	4
1/4	—	—	—	—	—	2	—	—	1	—	6	—
—†	10	—	—	1	—	—	10	—	—	—	2	6

* The fractions in this column are rough estimates obtained by simple inspection; lack of a sharp boundary between zones 3 and 4 precluded the use of exact methods of measurement.

† Zone 4 indistinguishable from zone 3.

The variations in the size of zone 4 are shown in Table 2. It shows that (*a*) in the newborn rats zone 4 was indistinguishable (Pl. 1, fig. 2), the abundant fat of zone 3 extending to the inner border of the cortex, (*b*) at 14 days zone 4 was at its broadest (Pl. 1, fig. 3), (*c*) after 14 days zone 4 became progressively narrower, (*d*) after 14 days zone 4 was on the average narrower in the females than in the males, (*e*) in the females zone 4 continued to narrow beyond 169 days and in the oldest group was usually indistinguishable, as in the newborn rats.

The variations in the fat content of zone 4 are shown in Table 3. It shows that (*a*) at 14 and 29 days fat was absent or virtually absent, (*b*) after 29 days fat was usually scanty (Pl. 1, fig. 4).

Fat in zone 4 was often least scanty in the cortical cells immediately outside the medulla. This phenomenon, henceforward referred to as the 'juxta-medullary fat concentration', was obvious in the following rats: at 84 days, three males (both adrenals of each) and six females (both adrenals of two, left only of three, right only of one);

at 169 days, one male (both adrenals) and six females (both adrenals of five, right only of one); after 169 days, three males (both adrenals of two, left only of one). The juxta-medullary fat concentration is not shown in any of our figures but is illustrated by Jackson (1919, Figs. 3–5) and Andersen & Kennedy (1932, Fig. 2, photographs 3 and 4).

Table 3. *Numbers of rats showing various amounts of fat in zone 4 at various ages (days)*

	Males						Females					
Fat in zone 4	<1	14	29	84	169	>169	<1	14	29	84	169	>169
Absent or virtually so	—	10	10	—	3	—	—	10	10	—	—	—
Scanty	—	—	—	9	7	10	—	—	—	10	6	4
Abundant*	10	—	—	1	—	—	10	—	—	—	4	6

* In inner part of cortex; zone 4 was indistinguishable from zone 3 except in two of the females aged 169 days.

DISCUSSION

The most striking age variation in cortical fat was the decrease in the amount of fat in the inner part of the cortex during the first 2 weeks of postnatal life and the gradual increase of fat in this region, a process appreciable at the end of the fourth week. The fat-free or virtually fat-free zone consisting of the inner half or more of the cortex 14 days after birth, which we have termed zone 4, is the region termed the juvenile cortex by Howard (1938). She found that the juvenile cortex resembled the mouse *X* zone in its time of appearance and in certain cytological respects, but that it differed from *X* zone in the absence of any sex difference in its duration or maximal size and also in the absence of degenerative changes; the juvenile cortex assumed the character of adult cortex by changes in the appearance and arrangement of its cells. Our observations show that the juvenile cortex of the rat resembles non-involuting mouse *X* zone in showing no obvious fat.

The juxta-medullary fat concentrations at first sight suggest cortical involution by fatty change of the type seen in the mouse *X* zone, but the invariable occurrence of the Schultz cholesterol reaction in such concentrations is a point against this suggestion; on the few occasions when the Schultz test has been applied to involution fat (Whitehead, 1934*a* (mouse *X* zone); 1935*b* (rabbit permanent cortex)) the result has been negative, although positive in the normal fat in the same adrenal. Since both normal and involution fat are sudanophil, the Schultz test is useful for distinguishing between them.

Our failure to find any relation between the appearance of cortical fat and the oestrous cycle conflicts with the results of Andersen & Kennedy (1932). According to these workers the characteristics of the oestrous fat pattern (re-expressed in our terminology) are (*a*) absence of zone 2, and (*b*) increased fat in zone 4 including the juxta-medullary fat concentration. Their Fig. 2, photograph 4, shows an adrenal from a rat in oestrus. Zone 2 is absent (we noted absence of zone 2 only once—from the left adrenal of one of the two rats whose oestrous phase was uncertain). Zone 4 contains more fat than in the dioestrous control (their Fig. 2, photograph 3), but the amount present seems well within the normal range for rats in dioestrus. As for the juxta-medullary fat concentration, differences in this exceeding that illustrated are common between the two adrenals of the same rat.

The variations in cortical fat in the rat may be compared with those previously observed in the mouse, guinea-pig and rabbit. The four species were alike in the following respects: (*a*) cortical fat was most variable in the inner part of the cortex, (*b*) fat in older animals was more abundant in the females than in the males, (*c*) the appearance of fat was closely similar in both adrenals of any individual, and (*d*) all normal fat always contained cholesterol. In other respects there were considerable species differences. The mouse and guinea-pig showed three fat zones: (1) a narrow subcapsular zone containing little or no fat, (2) a broad fat-laden zone, and (3) a broad fat-free or virtually fat-free zone surrounding the medulla. The rabbit showed only zones 1 and 2; fat in zone 1 was more abundant than in the mouse or guinea-pig, and zone 2 occupied all the remaining cortex at all ages. The rat was unique in two respects: (*a*) fat in zone 1 was far more abundant than in the other species, and (*b*) the narrow zone 2 of the rat was absent from the other species. Owing to the presence of the rat zone 2, zone 3 in the rat corresponds to zone 2 in the other species and zone 4 in the rat to zone 3 in the mouse and guinea-pig.

The four species also differed in the nature of the age variations. In the mouse fat usually occupied all the permanent cortex while the *X* zone was present; when the *X* zone had disappeared the amount of fat fell but soon showed no progressive change. In the guinea-pig there was a progressive decrease in the amount of fat from 14 days onwards. In the rabbit fat was relatively less abundant after the first month but there was no obvious progressive change. In the rat an early decrease in the amount of fat was followed by a progressive increase.

The results for the four species have a direct bearing on the conduct of experimental work on cortical fat. They provide standards for checking the normality of control material and make it possible to choose particular fat distributions for special study. The wide normal range of variation in the appearance of cortical fat within individual species makes it difficult to establish the occurrence of experimental changes; such a change might consist merely in a shift within the normal range, and it would then be impossible to say whether the fat in any particular adrenal had undergone a change. The close similarity in the appearance of cortical fat in the two adrenals of the same individual suggests that comparison of the two adrenals, one removed before experiment as a control, the other after experiment, would be the best method for detecting experimental changes. It has been found, however, that mere removal of one adrenal is followed by gross changes in the appearance of cortical fat in the adrenal left in situ (Whitehead, 1933*b* (mouse); Simmons & Whitehead, 1937 (guinea-pig); Stilling, 1888 (rabbit)); the only sound method is therefore to take a group of animals as the unit and to compare the frequencies of the various appearances of fat in the adrenals of control and experimental groups.

SUMMARY

1. The histological distribution of fat and cholesterol in the adrenal cortex has been studied in 120 normal rats at various ages from birth to over 169 days.

2. Fat was almost invariably abundant in the outer half of the cortex; the fat content of the inner half showed wide but regular variations with age.

3. At birth the inner half of the cortex was fat-laden; at 14 and 29 days it was fat-free or virtually fat-free; at later ages it usually contained fat, which was sometimes abundant.

4. The range of variation in the appearance of cortical fat was the same in both sexes.

5. The fat content of the inner half of the cortex was on the average higher in the females than in the males at all ages after 14 days.

6. The appearance of cortical fat was closely similar in both adrenals of any rat.

7. There was no evidence of any relation between cortical fat and the oestrous cycle.

8. All the fat in all the adrenals contained cholesterol, the distribution of cholesterol being the same as that of fat.

9. The results are compared with those previously obtained on the normal mouse, guinea-pig and rabbit.

10. The significance of the results for the experimental study of cortical fat is discussed.

The thanks of one of us (R. W.) are due to Prof. W. R. Bloor for the hospitality of his Department, to Prof. G. H. Whipple for the loan of apparatus, and to Dr Evelyn Howard (The Johns Hopkins University) for discussions. Both of us are indebted to Mr M. C. Orser (Rochester) for the photographs and to Prof. S. L. Baker (Manchester) for criticism.

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EXPLANATION OF PLATE 1

Figs. 1–4 show transverse frozen sections, stained with Sudan IV without counterstain, of adrenals of normal rats.

Fig. 1. Showing the four zones of the cortex: (1) a narrow black zone (fat-laden) below the capsule, (2) a narrow light grey zone (no fat obvious), (3) a broad black zone (fat-laden), and (4) a light grey zone (no fat obvious) occupying the inner third of the cortex. Medulla lighter grey than surrounding cortex. Right adrenal of a 65 g. female aged 29 days. ×60.

Fig. 2. Zones 1 and 2 less obvious than in fig. 1. Zone 3 (black, fat-laden) extends to the inner border of the cortex, zone 4 being indistinguishable. Cortex and medulla interdigitate. Right adrenal of a 5·5 g. female under 1 day old. ×100.

Fig. 3. Zones 1 and 2 as in fig. 1. Zone 3 is relatively narrower and zone 4 correspondingly broader than in fig. 1. Left adrenal of a 23 g. male aged 14 days. ×85.

Fig. 4. Pattern as in fig. 1, zone 4 occupying inner half of cortex. Zone 4 contains fat (dark grey) and therefore contrasts sharply with the medulla (almost white). The fat in zone 4 is less abundant than in zones 1 and 3, both of which appear black. Right adrenal of a 186 g. male aged 84 days. ×60.

ERRATUM

Vol. 76, Part 4, p. 346.

The correct magnifications of figs. 1–4 (Plate 1) are 41, 69, 58, and 41 respectively.

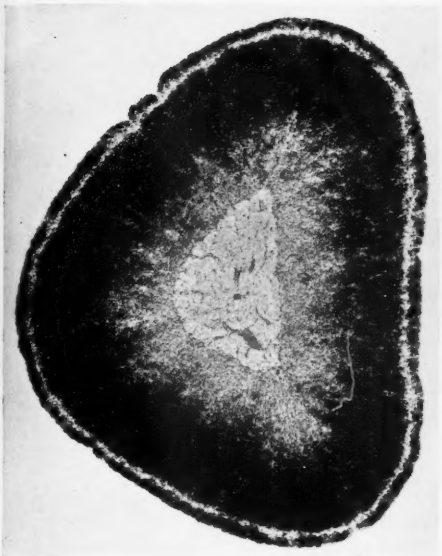

Fig. 1.

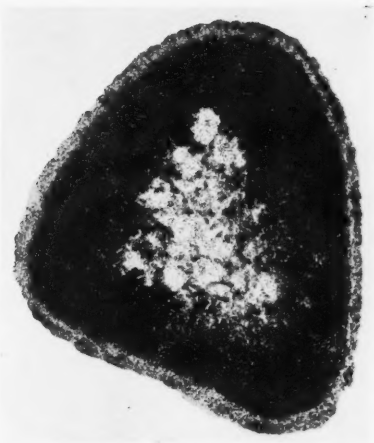

Fig. 2.

Fig. 3.

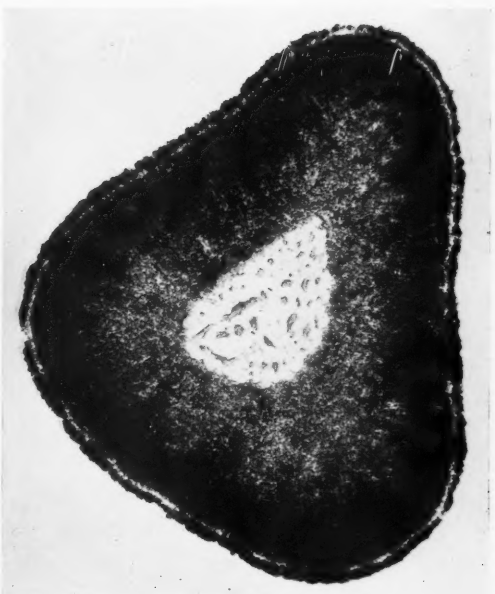

Fig. 4.

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VASCULARIZATION IN THE BRAINS OF REPTILES

IV. QUANTITATIVE STUDIES IN THE AMERICAN ALLIGATOR

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The previous paper of the present series (Craigie, 1941*c*) described some important features of the vascular architecture in the brain of *Alligator mississippiensis* Daudin, having reference both to the distribution of the superficial arteries and to the arterio-venous relations and the morphology of the capillary system within the brain substance. The latter were shown to have considerable possible phylogenetic significance. It remains for the present communication to report the results of measurements of the capillaries in various regions. Such measurements in the turtle have already been reported (Craigie, 1941*a*), but, as was pointed out in that connexion, the Crocodilia are rather far removed from the Chelonia phylogenetically and are considered to be more closely related to the reptilian ancestors of the birds while the Chelonia stand nearer to the line leading to mammals. Further, the circulatory system of the crocodilians is more advanced than that of other reptiles in respect of the separation of the ventricle into two chambers. Hence, if a comprehensive view of cerebral vascularization is to be attained, a knowledge of conditions in this order as well as in chelonians appears to be required.

Sterzi (1904) stated that in the alligator, as in chelonians, snakes, and a few lizards, the capillaries are more slender and form smaller meshes than in amphibians, but apart from this any comment upon the size or the richness of the capillaries in the central nervous system of any crocodilian appears to be absent from the literature.

MATERIAL AND METHODS

The material upon which the present report is based consists essentially of ten brains of young alligators ranging in length from 40 to 55 cm. The animals were procured alive from an alligator farm in Florida. All were males. Carmine gelatine was injected from the heart at pressures starting at 80 or 100 mm. of mercury and raised finally to 220–300 mm. The brains were fixed in 10% formalin, imbedded in celloidin, and cut in serial transverse sections. In some cases every eleventh section and in other cases every twelfth section was 200 μ thick, the remainder being 20 μ. When every twelfth section was thick, the one immediately following it was mounted separately and stained with cresyl violet. Series of sections in other planes than transverse were available but were not used for the measurements.

In addition to the above material, two animals, respectively 50 and 52 cm. long, were killed by injection of histamine into the heart. The brains were fixed and imbedded as above, cut in serial transverse sections 20 μ thick and stained with Weil's myelin stain, which coloured intensely the blood in the vessels and stained the walls faintly, so that the capillaries could be studied satisfactorily wherever the myelinated fibres were not too numerous. This method of staining was adopted in preference to the

benzidene technique because it had been found more satisfactory in some researches on the frog brain in progress in this laboratory. It turned out less well in the alligator than in the frog but was nevertheless fully adequate, with the limitation mentioned above.

Shrinkage in length, breadth, and height of the brain was measured in two injected specimens. Unexpected difficulty in identifying after sectioning some of the points which had been measured in the fresh brains caused the results to be possibly less accurate than had been anticipated, but an average linear shrinkage of approximately 20 % and a correction coefficient of 0·62 for length of the capillaries in a unit volume of tissue (Craigie, 1924) were obtained.

The instruments used for measurement of vascularity were the same as those employed in the writer's previous studies of this nature and, as in these, all measurements were made by the writer personally.

The regions selected for the measurements were considered to be the same which had been studied in the turtle. They were identified with the help chiefly of Crosby (1917), Crosby & Woodburne (1940), Huber & Crosby (1926), Kappers (1910, 1912), Larsell (1932), and Weston (1936). Other useful publications were Craigie (1936), Kappers (1934), Kappers, Huber & Crosby (1936), Kurotsu (1935), de Lange (1917), and Rose (1919, 1923).

OBSERVATIONS

As was recorded by Sterzi (1904), and in the previous communication of the present series, the capillary bed in the alligator's central nervous system is of the reticular type, forming a three-dimensional network continuous throughout the parts of this system. Differences in density of the spongy capillary net in various regions are obvious even to casual inspection, and regions of considerable vascular richness are to be noted in the diencephalon and in the mesencephalon. As has been pointed out by numerous authors who have studied various animals, such areas of markedly different vascularity usually correspond with distinct histological centres. There are, however, exceptions to this rule, as will be noted below.

Calibre of the capillaries

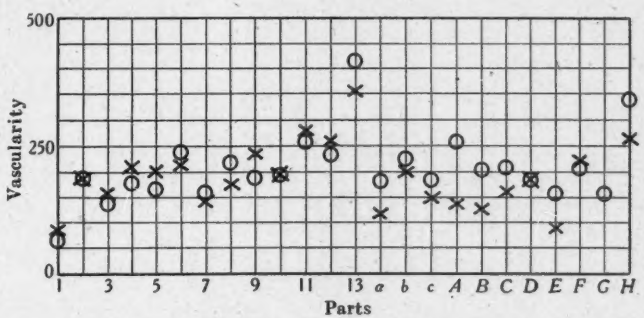
As in the case of the turtle, the average diameter of fifty capillaries, chosen at random, was determined in each of six regions (two in the medulla oblongata, two in the cerebellum, and two in the forebrain) in each of five specimens, and the results are presented in Table 2.

These figures show that the value of 8·2 μ previously published (Craigie, 1938*c*) on the basis of measurements in a single uninjected specimen was too high. The figures obtained in the different specimens were quite consistent, and such variations as occurred showed no relation to the pressure used in injection. In no case was there found a value so high as the one previously quoted.

Both the general average diameter and the values for the capillaries in the six different regions are markedly smaller than the corresponding figures for the turtle, being only about three-quarters of the latter. They are likewise smaller than the corresponding averages for the frog (Craigie, 1938*b*) or for other amphibians (Craigie, 1938*a*, 1941*d*) and agree fairly closely with those for the ratfish (Craigie, 1927), the monkey, and perhaps the horse (Craigie, 1938*c*).

It has been pointed out in previous communications (e.g. Craigie, 1941*d*) that the calibre of capillaries in material injected with gelatin usually averages less than that of blood-filled vessels in sections containing the latter. This is to be expected in view of the fact that in life the diameter of the capillaries is often less than that of the erythrocytes, the latter being squeezed but also distending the capillaries as they pass through them.

Hence, while the comparison of results obtained in injected material is probably permissible, they cannot be set directly beside uncontrolled values derived from stained sections. The erythrocyte of *Alligator mississippiensis* measures about $16{\cdot}5 \times 8{\cdot}0 \times 1{\cdot}8\,\mu$ after fixation and imbedding or about $20 \times 10 \times 2$ fresh (Craigie, 1941*b*), which is a little larger than the red cell of the painted turtle. Measurement of the capillaries in the hippocampus in four non-injected alligator brains gave the following uncorrected results: in a 6 ft. male, $9{\cdot}2\,\mu$; in a 7 ft. 4 in. female, $6{\cdot}6\,\mu$; in two 50 cm. males, 5·7 and $5{\cdot}1\,\mu$ respectively. The first of the latter two was killed with histamine, the second with chloroform, while the two larger animals were probably shot.



Text-fig. 1. Graph showing the relative vascularity in micra per 100^3 cu. μ of fresh tissue of parts studied in the brains of the alligator and the turtle. ○ alligator; × turtle. 1, fasciculus longitudinalis medialis; 2, nucleus motorius dorsalis X; 3, nucleus XII; 4, nucleus motorius VII; 5, nucleus motorius V; 6, nucleus fasciculi solitarii; 7, nucleus spinalis V; 8, cerebellum: molecular layer; 9, nucleus sensibilis principalis V; 10, cerebellum: granular layer; 11, cerebellum: lateral nucleus; 12, nucleus vestibularis ventrolateralis; 13, nucleus cochlearis magnocellularis. *a*, primordial nucleus supra-opticus; *b*, nucleus mamillaris lateralis; *c*, nucleus hypothalamicus ventromedialis. *A*, hippocampus (cell layer in H_3); *B*, parahippocampal cortex (cell layer); *C*, primordium neopallii (cell layer); *D*, primordium neopallii (molecular layer); *E*, pyriform cortex (cell layer); *F*, palaeostriatum; *G*, neostriatum (medial); *H*, neostriatum (lateral) ('core nucleus' in the turtle).

It may be noted in passing that even the largest measurement obtained is considerably less than the calibre of the corresponding vessels in *Sphenodon* (Craigie, 1941*b*).

The differences in calibre found in different regions are not great enough to be significant, but the somewhat large size of the capillaries in the hypoglossal nucleus receives confirmation from the fact that neither in the turtle nor in amphibians did any of the other centres examined have capillaries wider than those in this nucleus.

Richness of the capillary supply

The total length of the capillaries in a unit volume of tissue in the various parts examined is recorded in Table 1 and is represented graphically in Text-fig. 1. The probable errors of the averages are comparable with those found in the studies of other

Table 1. *Total length in μ of capillaries in* $1/2 \times 189^2 \times 200$ *cu. μ of tissue on the slide*

	Alligator (injected)										Average length in $1/2 \times 189^2 \times 200$ cu. μ	P.E. of average		Corrected average length in 100^3 cu. μ fresh tissue (coeff. 0·62).	Alligator (stained)	
	8 ♂	9 ♂	12 ♂	13 ♂	14 ♂	16 ♂	17 ♂	19 ♂	20 ♂	21 ♂		±	%		22 ♂	23 ♂
Fasc. long. medialis	511	450	453	369	311	293	397	338	277	413	381	16·46	4·3	66	—	—
Nuc. motorius X	1053	995	1034	1470	1004	1076	1177	1268	1046	1250	1137	32·58	2·9	197	815	756
Nuc. motorius XII	926	792	730	888	767	785	784	912	782	830	820	14·23	1·7	142	352	573
Nuc. motorius VII	907	1204	894	1205	951	1235	887	973	951	1172	1038	30·83	3·0	180	—	841
Nuc. motorius V	1020	917	922	916	1014	889	976	1024	1104	1077	986	15·73	1·6	171	536	—
Nuc. fasc. solitarii	1380	1310	1415	1828	1598	1515	1154	1231	1112	1532	1407	46·79	3·3	244	532	—
Nuc. spinalis V	787	1071	774	1024	879	924	938	800	880	1158	924	27·28	3·0	160	420	418
Cerebellum: molec. layer	1385	1273	1352	1430	1239	1342	1203	1185	979	1321	1271	27·60	2·2	221	614	1013
Nuc. sens. princ. V	1242	1044	973	1121	913	1108	963	1257	1164	1188	1097	25·64	2·3	190	464	681
Cerebellum: gran. layer	1147	940	1415	1213	1413	1036	1032	925	890	1461	1147	46·58	4·1	199	748	686
Cerebellum: lat. nucleus	1606	1449	1579	1505	1383	1416	1495	1674	1369	1548	1502	21·39	1·4	261	970	986
Nuc. vestib. ventrolat.	1514	1358	1446	1383	1340	1264	1344	1566	1222	1200	1364	25·48	1·9	237	—	939
Nuc. coch. magnocel.	2567	2150	2398	2799	2576	2011	2487	2469	2395	2325	2418	47·52	2·0	420	—	1055
Primord. nuc. supra-opt.	1043	972	952	1316	1114	1099	955	1006	1026	1121	1060	23·42	2·2	184	703	825
Nuc. mam. lat.	1122	—	1441	1562	1017	1442	1115	1512	1182	1441	1315	45·33	3·4	228	—	1023
Nuc. hypoth. ventromed.	—	953	961	1505	902	1166	889	1020	878	1343	1069	50·20	4·7	186	502	661
Hippocampus*	1351	1520	1643	1665	1426	1377	1633	1747	1221	1342	1493	37·07	2·5	259	732	994
Parahip. cortex†	1198	1134	1164	1119	1100	1199	1204	1261	1152	1277	1181	12·42	1·1	205	655	844
Prim. neopal. (cells)	1038	1154	1135	1343	1272	1247	1169	1301	1232	1329	1222	20·69	2·0	212	885	773
Prim. neopal. (molec.)	912	1137	995	1330	940	1002	934	919	1050	1326	1055	33·91	3·2	183	679	716
Pyriform cortex†	834	958	974	937	858	824	958	938	998	960	924	13·19	1·4	160	581	534
Palaeostriatum	1044	1041	1281	1359	1411	1294	1228	1039	1049	1348	1209	32·22	2·7	210	721	723
Neostriatum (medial)	809	957	814	942	1016	1046	750	916	776	1099	913	25·44	2·8	158	690	841
Neostriatum (lateral)	2354	1631	2056	1773	1936	1693	2247	1868	2238	1908	1970	37·59	1·9	342	1653	1343

* Middle cellular layer in area H_3 as designated by the writer in the alligator and in the bird (Craigie, 1935). † Cellular layer.

animals and are considered to be small enough to indicate reasonable trustworthiness of the results.

The relative poverty of the medial longitudinal bundle is obvious, as would be expected, and most of the other differences are statistically significant. The vascularity of the motor vagus nucleus, it is true, is not significantly different from that of the motor facial nucleus, the granular layer of the cerebellum, the primordial supra-optic nucleus, the primordium neopallii, or the palaeostriatum. Nucleus XII is significantly different from all other parts, but the motor VII nucleus does not differ to a significant degree from the motor V nucleus, the chief sensory V nucleus, the granular layer of the cerebellum, the two poorer hypothalamic areas, or the molecular layer of the primordium neopallii. Similar conditions exist in respect of the other regions, but the larger differences are all significant—including all those differences which separate each of the nucleus cochlearis magnocellularis and the lateral part of the neostriatum from other regions. As in the turtle, the difference between the layers of the cerebellar cortex is not significant, but that between the layers of the primordium neopallii is.

In general, the relative vascular richness of the various parts is quite similar to that in turtles, the richer hippocampal cortex being most divergent (Text-fig. 1). Also, the order of magnitude of the vascularity as a whole is quite close to that in the turtle, averaging only about 10 % higher, with the excess chiefly in the forebrain. This precise relationship between the species, of course, would be considerably affected by any error in the very approximate coefficients used to correct for shrinkage.

The hypoglossal nucleus is the least vascular of the grey matter.

The outstanding richness of the cochlear nucleus agrees with what has been found in the other animals examined. It should be pointed out, however, that the lateral portion of the nucleus, in which the measurements were made, is visibly richer than the medial portion. In passing, it may be noted further that the related nucleus laminaris is likewise very richly vascularized, and in it the capillaries are situated chiefly in the non-cellular matter within which the plate of cells is imbedded rather than in immediate relation with the perikarya (Text-fig. 2). The superior olivary nucleus also stands out in sections of injected material by reason of the number of capillaries within it. It is interesting that, while the magnocellular nucleus is richer than in the turtle, it is just about equal in vascularity to that of the frog (if the shrinkage correction coefficients used are not obscuring a real difference).

As in the turtle, and in contrast with conditions in mammals, the hypothalamus is in no part very richly vascular. It has, however, progressed considerably from the poverty which characterizes it in *Necturus* (1940*b*). The best supplied of the hypothalamic sites examined is the lateral mamillary nucleus, which has a capillary density equal to that in the dorsal cochlear nucleus. The supra-optic nucleus, which in mammals is so remarkably rich, is represented by somewhat scattered cells (Crosby & Woodburne, 1940), and the capillaries in relation with these are evidently less numerous than those in the adjacent periventricular grey matter or in nucleus '*a*' of Röthig as illustrated by Huber & Crosby (1926).

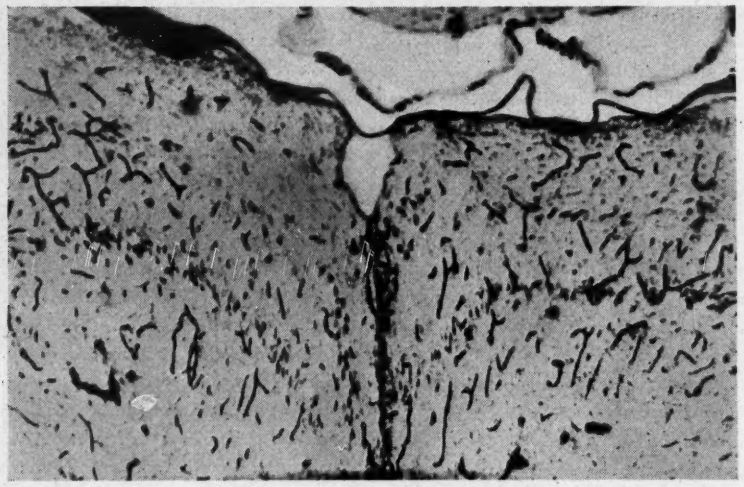
It is probably significant that, in the cerebral hemisphere, all the cortical situations in which measurements were made, with the exception of the molecular layer of the primordium neopallii, are definitely more vascular than in the turtle.

The palaeostriatum is represented by the 'ventrolateral- large-celled area' of Crosby (1917), which she considered to correspond with the 'lentiform nucleus' of

Johnston in the turtle. In what appeared to be this area, the vessels look more numerous laterally than medially and most measurements were made in the lateral part, the results being comparable with those in the region of the turtle specified.

On the other hand, identification of a homologue of the basal amygdalar nucleus of the turtle was considered too uncertain for any measurement to be made to represent this part.

In the neostriatum, there appeared a most conspicuous example of a vascular difference which could not be seen to correspond with any histological differentiation. The lateral part of this mass contains an area of conspicuous capillary richness contrasting markedly with the remainder, as may be seen in Pl. 1, figs. 1, 2. Measurements revealed that this lateral portion of the neostriatum was surpassed in vascularity only



Text-fig. 2. Photomicrograph of the region of the laminar nuclei in a transverse section 20 μ thick stained with cresyl violet after injection with carmine gelatine. $\times 50$.

by the magnocellular cochlear nucleus and was approached by no other, while the medial portion was on a level with the pyriform cortex (the poorest of the forebrain situations) and the spinal nucleus of the Vth nerve. In the turtle, the neostriatum contains a distinct 'core nucleus' which is richer than the rest and in which the measurements were made. The value for the latter is plotted at *H* in Text-fig. 1 and may be seen to lie between the values for the medial and lateral parts in the alligator.

Since the injection method has been subjected to some criticism, while the greater reliability of measurements based upon sections of material in which the blood is retained in the capillaries and appropriately stained, especially after killing by histamine, has been maintained (Lindgren, 1940), two alligators were prepared by this method, as already stated.

In both these brains, certain areas were visibly incompletely filled and so were not suitable for the present purpose. In the remaining situations which had been studied in the injected material the vessels appeared to be adequately revealed and measurements were made. The results are set down in the last two columns of Table 1, without

correction of any kind. It may be observed that they are in every case lower than the values obtained from the injected material and so give no hint that the latter are not reliable. They themselves, on the contrary, being so consistently low, appear too untrustworthy to be considered, and consequently the averages, the graph, and the foregoing discussion are based entirely upon the injected specimens, which are believed to be adequate.

Volumes of the capillaries and areas of their walls

The volumes and surface areas of the capillaries in the six regions in which the diameters were measured are listed in Table 2. In three of these six regions the total length of the vessels in a unit volume of tissue is less in the alligator than in the turtle, so that, since the diameters are less in all cases, the volumes and areas are also necessarily considerably smaller. Of the three remaining regions, only the hippocampus has sufficient length of capillaries to make either volume or area surpass, or even approach, that in the chelonian.

Table 2. *Average diameters, surface areas, and volumes of gelatin-injected capillaries in various parts of the brain of the alligator*

	Diameter in μ, not corrected	Diameter in μ, corrected for shrinkage	Area in sq. μ of capillary walls in 100^3 cu. μ of fresh tissue	Volume in cu. μ of capillaries in 100^3 cu. μ of fresh tissue	Area in sq. mm. to which 1 cu. mm. of blood is exposed
Fasc. longitudinalis medialis	4·4	5·5	1140	1568	727
Nucleus XII	4·6	5·8	2587	3752	690
Cerebellum, molecular layer	4·2	5·3	3680	4874	755
Cerebellum, granular layer	4·3	5·4	3401	4558	741
Palaeostriatum, ventrolat. large-celled area	4·5	5·6	3694	5172	714
Hippocampus	4·4	5·5	4475	6152	727
Average	4·4	5·5			726

In the medial longitudinal bundle, both volume and area of capillaries are smaller even than in the frog; in the hypoglossal nucleus the volume is again smaller but the area is about the same as in this animal. In the granular layer of the cerebellum the volumes of the capillaries do not differ very greatly in frog and alligator. The remaining values for the alligator are intermediate between those for frog and turtle.

In the hippocampus, the area of the walls is intermediate between that in the turtle and that in the rat, while the volume is greater than in either.

The area of wall to which a unit volume of blood is exposed (not allowing for the stretching of the capillaries by the blood cells as noted above) is shown in the last column of Table 2. Being a direct derivative of the radius of the vessels, this area is larger than in the turtle and larger even than in the frog (which surpasses the turtle in this particular) except in the molecular layer of the cerebellum, where it is about the same as in the frog.

Thus partial compensation for the smaller volume of blood in the brain tissue of the alligator as compared with the turtle is seen in the spreading of this blood more thinly over the endothelial surface. Since the surface area is, nevertheless, also smaller than in the turtle, the system as a whole must be regarded as having less capacity even if it is more economical. The formation of more slender vessels may be a step in the direction taken by the ancestors of the warm-blooded animals, but it has not yet gone

far enough to enable the alligator to metabolize as rapidly even as the turtles. This fits in with the physiological observations of Benedict (1932) who showed that the metabolic rate in the alligator (in terms of unit surface area of the body) is comparable with that in snakes and lizards, while the rate in the turtles is higher.

SUMMARY

1. The central nervous organs of the alligator have a continuous spongy reticular vascular bed in which localized differences in density are obvious. The areas of markedly different density, while they usually correspond with histologically differentiated centres, do not invariably do so.

2. The calibre of the capillaries is smaller than in the turtle or in amphibians, but corresponds fairly closely with that in the ratfish and with that in the monkey.

3. The relative vascularity of the various parts is similar to that in turtles, the richer hippocampal cortex being most out of line.

4. The magnocellular cochlear nucleus is the richest part found and the hypoglossal nucleus is the poorest grey region.

5. The magnocellular cochlear nucleus is visibly richer in its lateral part than medially; the same is true of the ventrolateral large-celled area of the palaeostriatum, and a conspicuous area of great richness appears within the lateral portion of the neostriatum.

6. The hypothalamus is in no part very vascular, the richest portion observed being the lateral mamillary nucleus.

7. Most parts of the cerebral hemisphere, particularly the cortical areas with the exception of the molecular layer of the primordium neopallii, are definitely richer than in the turtle, but for the brain as a whole there is only about 10 % excess over the vascular supply in the latter animal.

8. The volumes and surface areas of the capillaries are in many parts less than in the turtle, though the area exposed to a unit volume is greater. Hence, while the more slender vessels constitute presumably a more efficient system, the total functional capacity of the system would appear to be less, a fact in keeping with the recorded lower metabolic rate of the alligator.

9. Measurements of the capillaries in two animals killed with histamine yielded everywhere smaller values than those obtained from injected specimens.

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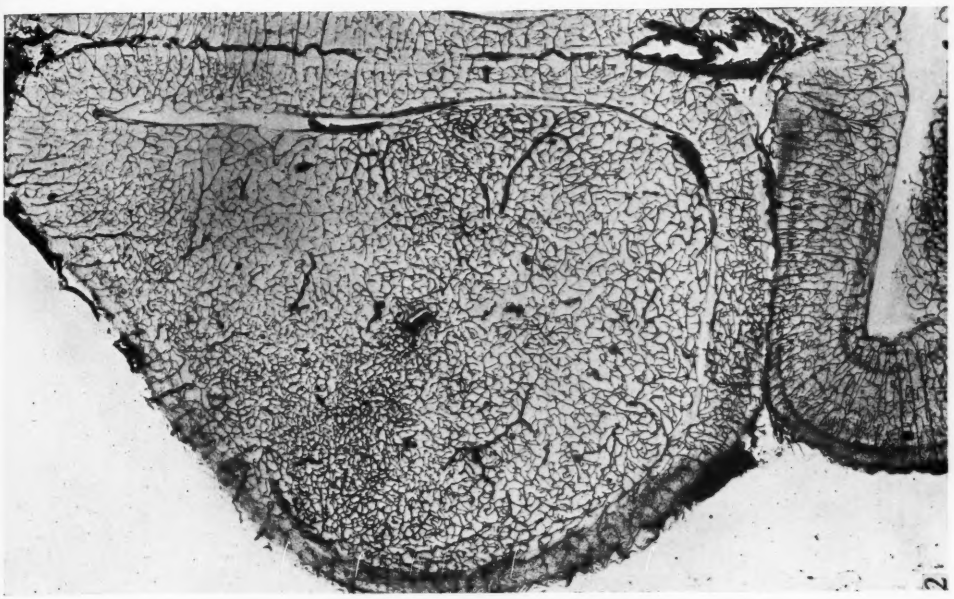

Fig. 2.

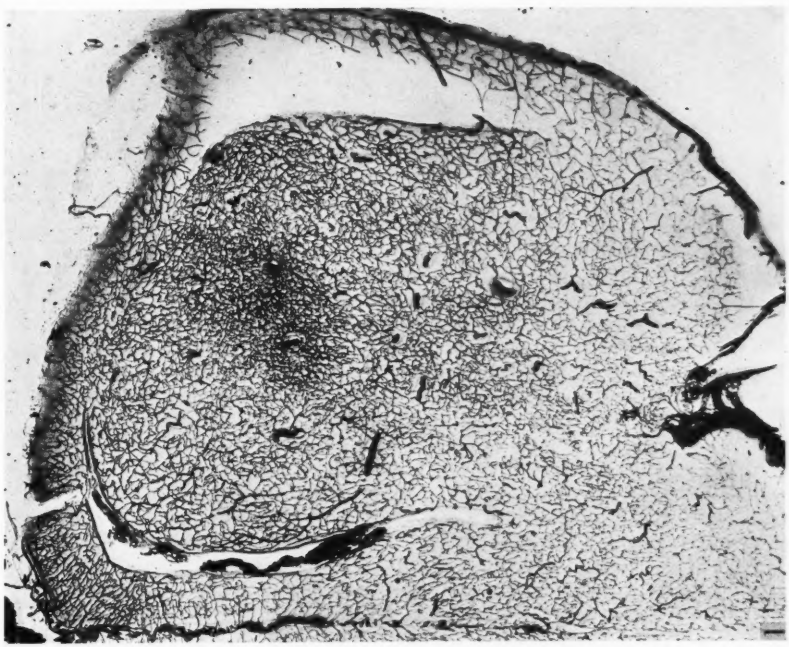

Fig. 1.

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EXPLANATION OF PLATE 1

Fig. 1. Transverse section, 200 μ thick, of right cerebral hemisphere of alligator injected with carmine gelatine, $\times 8$. The richly vascular area in the lateral part of the neostriatum stands out conspicuously.

Fig. 2. Coronal section, 200 μ thick, of left cerebral hemisphere of alligator injected with carmine gelatine, $\times 8$. The richly vascular area in the lateral part of the neostriatum is evident. Medial and slightly caudal to it another relatively rich area extending to the medial surface of the corpus striatum is Rose's field L.

THE MID-DORSAL HAIR WHORL IN AN AUSTRALIAN OF EUROPEAN ANCESTRY

By F. FENNER, A.A.M.C.

The occurrence of a centripetal whorl in the dorsal hair tracts of man was first described in 1927 by Wood Jones in foetuses of mixed ancestry from Hawaii. Later it was found to occur in a high percentage of pure-blood Australian aborigines (61% in a Victorian group (Wood Jones, 1934); 43% in a Central Australian series (Gray, 1934)). As far as can be ascertained, this dorsal whorl, with the reversal of hair tracts in the lower dorsal region which usually accompanies it, has never been described in Europeans. During routine clinical examinations in a medical unit of the Australian Imperial Force, a patient, A.T.T., was noticed to have a centripetal dorsal hair whorl. His hair tracts were fully mapped out and are described below.

The man, aged 19 years, was born in Melbourne, Victoria. His parents and grandparents were born in Australia, except his maternal grandfather, who was French. As far as could be ascertained, most of his great-grandparents were English or Scotch. Physically he was fair-haired and blue-eyed, with no features suggesting anything but a European ancestry.

Figs. 1 and 2 show the condition of the hair tracts of the trunk. Deviations from the European normal were seen in several areas, as described below.

Dorsal region of trunk. In the lower dorsal region, extending from the level of the spine of D10 to the spine of L2, there was a symmetrical reversal of the hair tracts so that they ran cephalad. All Wood Jones's Victorian aborigines, and 43% of Gray's Central Australian series, showed this reversal. In the region of the spine of D10, where the dorsal cephalad stream met the normally directed tracts from the upper trunk, there was a centripetal anti-clockwise spiral. The type of hair tract seen on the dorsal aspect of the trunk of this European is thus identical with that described by Gray and Wood Jones.

Posterior aspect of thigh. In the upper third of the posterior aspect of both thighs there was a reversal of the hair tracts which thus sloped upwards and medially towards the coccyx. The parting line between this upward stream and the normal caudad stream was quite definite. Gray found this reversal in every case in his Central Australian series, though it has not been otherwise mentioned in the literature on human hair tracts.

Lateral abdominal parting line. There was a bilateral reversal of the lateral abdominal feathering. Diverging tracts began in centrifugal whorls situated on either side just below the level of the umbilicus, and ascended to the infra-axillary region where they met the tracts descending from the axillary centre. Gray found a similar condition in three subjects of his Central Australian series.

Anterior aspect of thigh. Apart from the disturbance caused by the reversal of the anterior abdominal parting line and centrifugal whorls in the lumbar regions, the tracts on the anterior aspect of the thighs were normal.

Anterior aspect of arm. The anterior aspects of the arms showed slightly different patterns on either side. On both sides there was a feathering just below the axilla

and a divergence line just above, but on the right side these streams merged while on the left a clockwise centrifugal whorl was interposed between them.

Occipital whorl. The occipital whorl was clockwise and situated to the right of the mid-line. The head parting line was on the left.

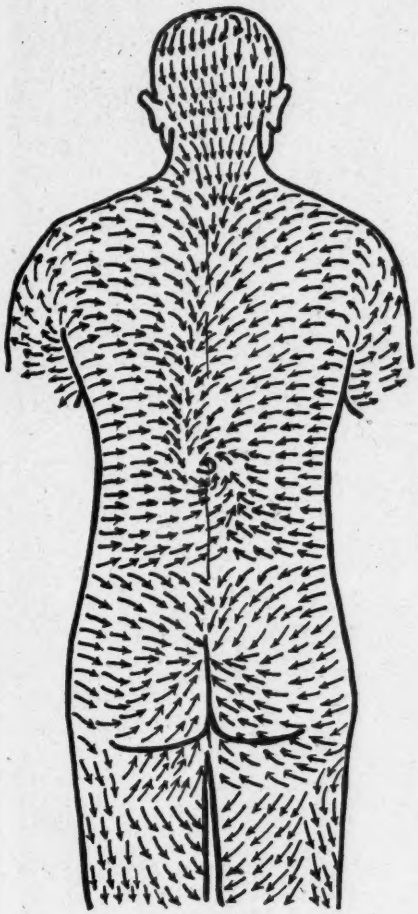

Fig. 1.

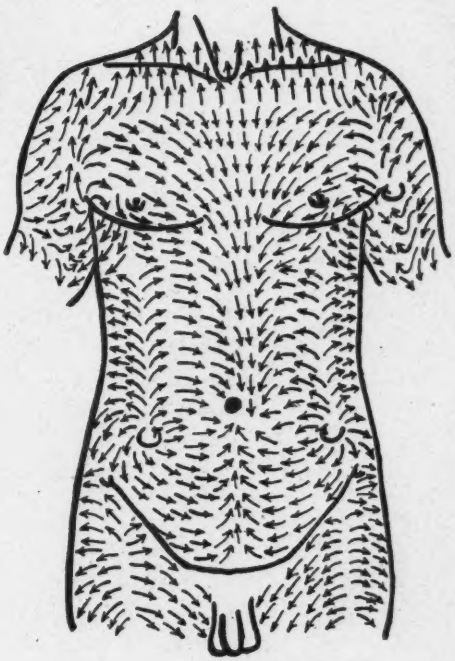

Fig. 2.

Fig. 1. Dorsal view of trunk of subject A.T.T., showing hair tracts. Note dorsal spiral, dorsal reversal and posterior thigh reversal.

Fig. 2. Anterior aspect of trunk of subject A.T.T., showing hair tracts. Note reversal of lateral abdominal partings, and centrifugal whorls in lumbar regions.

Pinna. There was a hair parting line along the line of attachment of the pinna. The hair streams on the pinna flowed down around the upper part of the helix and up around the lobule and lower helix to meet in the crest opposite the tragus.

Other regions of the body showed no deviation from the accepted normal for Europeans (Kidd, 1903; Wood Jones, 1929).

SUMMARY

The occurrence of several anomalies of the hair tract arrangement, including a mid-dorsal whorl and lower dorsal reversal, is reported in an Australian of unmixed European ancestry.

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SPECIALIZED CONDUCTING TISSUE IN THE HEART OF THE GOLDEN HAMSTER (*CRICETUS AURATUS*)

By E. W. WALLS, *Department of Anatomy, Glasgow University*

This investigation comprises part of a detailed study of the specialized conducting tissue in the rodent heart.

Technique. The hearts of animals freshly killed by coal gas were removed with lengths of the great vessels attached and were perfused thoroughly with normal saline. The histological technique used was the modification of the pyridine-silver method used by Blair & Davies (1935), with adjustment of times to suit the size of the heart. Serial sections of two entire hearts were cut at 10μ and every second section mounted. Four additional hearts were cut and stained as follows: two with haematoxylin and eosin, one with Van Gieson and one with Mallory, but these stains were not as useful for the differentiation of specialized conducting tissue as the pyridine-silver method. In an attempt to obtain better staining of the cardiac muscle fibres generally, and of the specialized fibres in particular, several animals were fed for 10 days prior to death on a diet calculated to increase the glycogen content of the cardiac muscle (Davies, 1930). The result was disappointing. The construction of a model of the entire heart by the wax-plate method helped considerably in placing with accuracy the different parts of the conducting system.

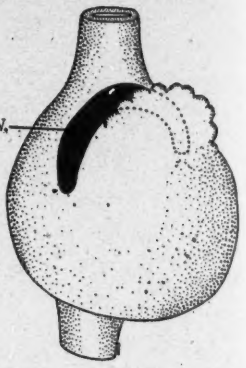
GENERAL DESCRIPTION OF HEART

Certain features of the hamster's heart, with reference to its general structure and functional activity, are of interest. As regards structure, there are, opening directly into the right atrium, the following vessels: right and left superior venae cavae, the vena azygos and the inferior vena cava. The orifices of the left superior vena cava and vena azygos are each guarded by two valves, and that of the inferior vena cava by one. Apparently, therefore, parts of the left venous valve have persisted, as well as those portions of the right venous valve which are usually recognizable in the adult mammalian heart. As regards function, the heart rate, determined electrically, is 450 per minute, the average body weight of this breed of hamster being about 100 g. The electrocardiograms were taken with the animal under ether anaesthesia, from lead 1, i.e. with both front paws placed in saline-filled paraffin blocks.

Specialized Tissue in the Heart

The sinu-atrial node. General position and size: It is a large structure shaped rather like a horseshoe, lying in relation to the upper part of the sulcus terminalis, and forming a wide ∩ above and in front of the opening of the right superior vena cava into the right atrium (Text-fig. 1, *S.A.N.*). The highest part of the node actually lies in the antero-lateral wall of the vein. The anterior or medial portion of the node, hereafter referred to as the anterior limb, extends downwards behind the aorta and immediately to the right of the anterior part of the atrial septum; in this portion of the septum at a lower level lies the atrio-ventricular node. The posterior or lateral limb descends for some

distance in the sulcus terminalis, reaching to a lower level behind than the anterior limb in front. It does not, however, extend as far caudally as the right sinus valve guarding the opening of the vena azygos. In section the node is seen to occupy the entire thickness of the heart wall, and it is readily identified by its compact appearance compared with the neighbouring atrial and sinus muscle, and by the greater thickness of the endocardium under it (Pl. 1, fig. 1). The greater part of the node lies to the sinus side of the sulcus terminalis, but that portion which lies in the roof of the atrium, and which therefore connects the two limbs of the node, does so in the substance of the crista terminalis.



Text-fig. 1. Diagram of heart from right side to show sinu-atrial node (*S.A.N.*) in situ. The anterior limb of the node is hidden by the atrial appendage.

Histology of the sinu-atrial node (Pl. 1, fig. 2). The node is composed almost entirely of muscle fibres, but a small amount of fibrous tissue is present around the extremity of its anterior limb. The muscle fibres are mainly of two distinct types, small and large. The small fibres are not unlike ordinary atrial fibres except that they are on an average slightly smaller, 5 μ in thickness compared with 6·5 μ, and also that they do not exhibit well-marked transverse striation. The large fibres, which are more than three times the size of atrial or sinus fibres—being 20–22 μ in diameter—possess a very pale-staining cytoplasm in which there is a large, round, centrally placed and, with silver, lightly coloured nucleus. Transverse striation can be demonstrated in these large fibres but in none of them is it obvious. There are, therefore, in the sinu-atrial node, fibres of the nature of the so-called Purkinje fibres. As far as I have been able to discover from a study of the literature such fibres have not previously been described as occurring in this situation. Several authors have described collections of Purkinje fibres in the right atrium of the mammalian heart, but for the most part no mention is made of such fibres having any connexion with the sinu-atrial node. However, Davies (1930), in the avian heart, Blair & Davies (1935), in the ox heart, and Pannier (1939), in the cat, all describe continuity between the fibres of the sinu-atrial node and Purkinje fibres. In the hamster's heart here described, large typical Purkinje fibres occur actually in the sinu-atrial node as part of its substance. The general direction of the nodal fibres is along the line of the sulcus terminalis, so that sections through the base of the heart cut the fibres along their long axis, and sections farther down the sulcus cut across them at right angles. It is quite certain that there is direct continuity between nodal fibres and the atrial and sinus musculature; no evidence could be found of any septum between the two types of fibre. The atrial wall around the site of entry of the left superior vena cava was examined closely for nodal fibres but none were seen. Moreover, no Purkinje fibres occur in the walls of either atrium other than those present in the sinu-atrial node.

Before leaving the description of the sinu-atrial node, mention must be made of a dense compact band of ordinary atrial muscle fibres which occurs constantly on the posterior wall of the right atrium, in line with but below the lower limit of the posterior limb of the node. It is not part of the conducting system but simply a specially strengthened portion of the heart wall.

The atrio-ventricular node. This is a well-marked structure of considerable size situated on the right side of the anterior part of the atrial septum immediately behind the aorta. In a general way it is spindle-shaped, the upper end of the spindle approximating closely to the lower end of the anterior limb of the sinu-atrial node. It is composed of two parts (Pl. 1, fig. 4):

(*a*) A more deeply placed portion made up of interlacing fibres which form a meshwork of a fairly loose nature. These fibres, which are muscular, are practically indistinguishable from atrial fibres, being between 6 and 7μ in diameter and showing complete transverse striation. Interspersed among the fibres of this part of the node are numerous blood vessels and a quantity of connective tissue. Near its lower end the atrio-ventricular bundle arises from it.

(*b*) A large compact bundle of fibres, subendocardial in position, the individual fibres of which are more lightly staining than those of the looser portion: in each fibre there is a large, pale, centrally placed nucleus. These fibres run longitudinally, and many of them are of quite large size, 10–16μ in diameter, approaching in appearance typical Purkinje fibres. This compact portion reaches to a higher level in the atrial septum than the loose portion, of which it appears to be a specialized upward extension.

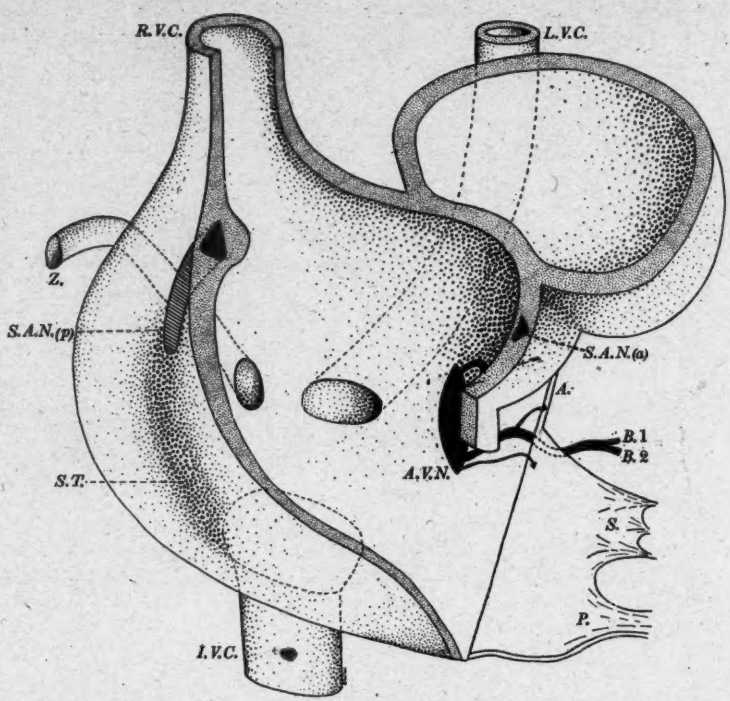
The more deeply placed, looser portion of the node becomes continuous with both right and left atrial muscle fibres, with the atrio-ventricular bundle, and, in places, with the more compact part of the node. The latter, on the other hand, can only be traced into continuity with a few right atrial fibres.

There are two prolongations from the atrio-ventricular node. The first passes from the upper part of the node forwards and to the right into the anterior wall of the right atrium towards, but not reaching, the anterior limb of the sinu-atrial node. There is no continuity between the nodes. The second prolongation arises from the lower part of the atrio-ventricular node and, running parallel with the right limb of the atrio-ventricular bundle, ends in the base of the septal cusp of the right atrio-ventricular valve.

The atrio-ventricular bundle. This structure arises from the lower portion of the atrio-ventricular node and arches upwards slightly as it passes forwards in the right trigonum fibrosum immediately behind and to the right of the aorta. When it reaches the muscular septum it divides into a right and left limb which straddle the septum (Pl. 2, figs. 5, 7). In its course it runs under the septal attachment of the septal cusp of the right atrio-ventricular valve, into which cusp, as already mentioned, a slip passes from the atrio-ventricular node. Arising from the upper aspect of the bundle, between its origin and where it passes deep to the septal cusp, there is a slender slip of fibres which passes into the anterior cusp of the tricuspid valve. This cusp has a considerable amount of ordinary atrial muscle in its substance. These slips of specialized tissue which pass into the valve cusps are similar to the single slip found in the avian heart by Davies: in the bird a branch of the right limb of the atrio-ventricular bundle passes into the muscular right atrio-ventricular valve at its anterior septal attachment, and Davies suggests that, ventricular systole being of such short duration in the exceedingly rapid hearts of small birds, the valve actively contracts early in ventricular systole in order that the greater part of the ventricular blood may be transmitted through the pulmonary artery. This suggestion could be made equally with reference to the hamster heart and, if correct, is a good example of this particular adaptation of structure to function in two different classes of vertebrates. No other branches apart

from the single slip into the valve cusp arise from the atrio-ventricular bundle before its division. This is quite different from what obtains in the rabbit: in that rodent (Lloyd, 1930) the atrio-ventricular bundle gives off early down-going branches prior to its division into right and left limbs. These early branches either penetrate the interventricular septum or pass downwards just under the endocardium of the left ventricle.

In the hamster the atrio-ventricular bundle, which is surrounded by a connective tissue sheath, is composed of fibres all of which are paler than the neighbouring heart

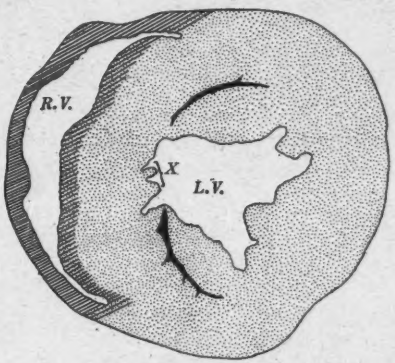


Text-fig. 2. Diagram of right atrium opened to show the specialized tissue. The left atrium is also represented. Note the two prolongations from the atrio-ventricular node into (1) the septal cusp of the tricuspid valve, and (2) the anterior wall of the right atrium close to the anterior limb of the sinu-atrial node. Also note the slip from the atrio-ventricular bundle into the anterior cusp of the tricuspid valve. *R.V.C.* right superior vena cava; *L.V.C.* left superior vena cava; *I.V.C.* inferior vena cava; *S.A.N.* (*a*), anterior limb of the sinu-atrial node; *S.A.N.* (*p*), posterior limb of the sinu-atrial node; *Z.* vena azygos; *S.T.* sulcus terminalis; *A.V.N.* atrio-ventricular node; *A.* attached margin of anterior cusp of right atrio-ventricular valve; *S.* septal cusp of right atrio-ventricular valve; *P.* posterior cusp of right atrio-ventricular valve; *B.* 1, left limb of atrio-ventricular bundle; *B.* 2, right limb of atrio-ventricular bundle.

muscle. Many of the fibres are large, up to 20μ in diameter, and similar to typical Purkinje fibres; transverse striation is not pronounced (Pl. 2, fig. 8). The right limb of the bundle can be traced from the bifurcation of the main bundle over the ventricular septum vertically downwards under the endocardium of the right ventricle. After a short course in which it spreads out slightly in relation to the right atrio-ventricular valve cusps, it seems to disappear by becoming continuous with the adjacent myocardium in the upper part of the interventricular septum. No Purkinje fibres whatever are

to be seen in the wall of the right ventricle. The presence of a moderator band was not positively established, but what was taken to be it, as well as several other slender muscular slips which crossed the ventricular cavity at intervals, were examined carefully for Purkinje fibres with negative results. Moreover, in none of these strands was there any special 'spread-out' of the fibres on reaching the free wall of the ventricle. The myocardial fibres in the wall of the right ventricle are distinctly smaller in diameter than those on the inner part of the left ventricle, and remain so right to the apex of the heart.

The left limb of the atrio-ventricular bundle follows a very short course down the left side of the septum and remains in a buried position. Its fibres are here and there continuous with the myocardium. A little below the level of disappearance of the left limb Purkinje fibres appear, first in the ventral wall and then a shade lower down in the dorsal wall of the left ventricle. In each position the Purkinje fibres spread out forming curved bands (Text-fig. 3). The dorsal band is situated half-way between the endocardial and epicardial surfaces, and at its upper end its medial or septal extremity is vertically in line with the left limb of the atrio-ventricular bundle but separated from it by an interval of ordinary myocardium. The question of continuity between the left limb of the bundle and the dorsal band of Purkinje tissue was gone into very thoroughly; the sections between that in which the left limb ceased to be recognizable and that in which the dorsal band of Purkinje tissue appeared were scrutinized repeatedly, but no continuity through the medium of structurally specialized muscle fibres could be established, the only connexion being by what seemed ordinary undifferentiated ventricular muscle fibres. The ventral band appears first in the depth of the ventricular wall as a short strand connecting the adjacent attached bases of two papillary muscles; lower down it expands medially and laterally. Traced downwards, these bands of Purkinje fibres (which form curved plates of considerable vertical extent) reach the endocardial surface of the ventricle on the septal side, and at a lower level their septal horns join in the subendocardium. The Purkinje fibres of these bands pass into obvious continuity with the adjacent myocardium. Below the point of fusion of the two bands, part of the endocardial surface of the left ventricle remains carpeted with these Purkinje fibres which towards the apex of the ventricle send a few false tendons across the cavity which here narrows markedly. Below this level the cavity again expands and the fibres in the inner part of the left ventricle become noticeably smaller. The cavity ends with an interlacement of ordinary myocardial strands, but close to the apex of the ventricle a few Purkinje-type fibres are still present in the free wall which here has become much thinner.



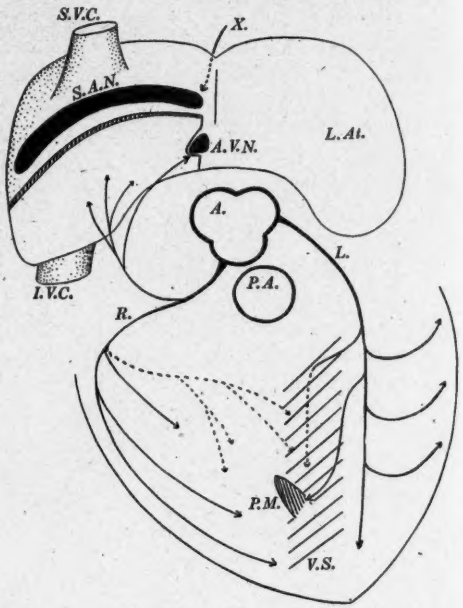
Text-fig. 3. Outline (simplified) of transverse section of ventricles, to show position of the buried plates of Purkinje fibres. Lower down the septal edges of the plates fuse in the region marked *X*. ×20. *R.V.* right ventricle; *L.V.* left ventricle.

In general the left ventricular wall is exceptionally thick—as much as six times the thickness of the free wall of the right ventricle—and throughout the internal half or

more of its extent it is composed of fibres noticeably broader than those in the external part of its wall or in any part of the wall of the right ventricle.

Surrounding each plate of Purkinje fibres in the wall of the left ventricle there is a clearly marked space (Pl. 2, fig. 9), which recalls the 'constant bursa' round the bundle of His described by Curran (1909).

Blood supply to the conducting tissue. The sinu-atrial node is supplied by an artery which appears to arise, not from either coronary artery, but from some other vessel in the mediastinum (Text-fig. 4). It approaches the node by entering the posterior wall of the atria, and runs ventrally in the atrial septum to reach the anterior horn of the node. From it branches pass into the node and constitute its entire supply. But no large artery runs longitudinally through the node as is described in many mammals and also in the avian heart (Davies, 1930).



Text-fig. 4. Diagram of coronary artery distribution. *R.* and *L.* right and left coronary arteries; *A.* aorta; *P.A.* pulmonary artery; *L.At.* left atrium; *A.V.N.* atrio-ventricular node; *S.A.N.* sinu-atrial node; *S.V.C.* superior vena cava; *I.V.C.* inferior vena cava; *V.S.* ventricular septum; *P.M.* anterior papillary muscle of right ventricle; *X.* artery to sinu-atrial node.

The atrio-ventricular node is supplied by branches of the right coronary artery, but the two parts of the node differ in their blood supply, the deeper looser portion being very much more vascular than the compact subendocardial part.

Although the limbs of the atrio-ventricular bundle could be followed only for a short distance, the blood supply to the septum is such that it appears certain that the left limb is supplied mainly by the left coronary artery and the right by both arteries.

The atria in this heart receive a very scanty blood supply. This is no doubt because, having such thin walls, their contained blood proves sufficient for their needs, but it does emphasize the significance of the special arteries to the nodes.

Anomalous aortic recess. In the posterior wall of the aortic root there is present a vertical fold which produces a longitudinal recess of varying width. It extends above and below the level of the aortic valve cusps and is undoubtedly a permanent formation. At its upper limit the recess is a narrow cleft and in transverse section appears to be the commencement of a third coronary artery arising from between the two posterior semilunar valves (Pl. 2, fig. 6). That this is not so is seen as the aortic stem is traced downwards, when the recess becomes very wide (Text-fig. 5). The wall of the recess is modified in structure; it is much thinner than the rest of the aortic wall and does not seem to contain the usual elastic tissue. Closely related to its external surface is the

atrio-ventricular node, and in fact some of the nodal fibres are attached to its wall. Through this connexion changes of pressure within the aorta will be instantly communicated to the node. The degree to which this anomalous aortic recess is developed is not constant, but, to some extent at least, it was present in all hearts examined.

Nerve cells and nerve fibres in relation to the specialized tissue. The cardiac ganglia in *Cricetus auratus* are found only in relation to the atria, this being in accordance with the findings of Meiklejohn (1913*b*) in the rat. The main ganglia are as follows:

(1) A small ganglion in the anterior wall of the superior vena cava above the level of the sinu-atrial node.

(2) Two large ganglia in the inter-atrial septum. The first is in the posterior part of the septum on a level with the upper portion of the sinu-atrial node but situated some distance behind it. The second ganglion is in the lower part of the inter-atrial septum a little above the level of the main part of the atrio-ventricular node, and from it numerous fibres can be traced into the node. This ganglion is a continuation downwards of a more cephalad ganglion found on the posterior wall of the left atrium.

Text-fig. 5. Three diagrammatic transverse sections through the aortic root, uppermost section on the left. *A.A.R.* anomalous aortic recess; *R.* right coronary artery; *L.* left coronary artery.

(3) Three ganglia in relation to the left atrium: (*a*) A large ganglion placed in front of the anterior wall of the left atrium behind the bifurcation of the pulmonary artery. (*b*) A large ganglion in the posterior wall of the left atrium extending downwards for about the middle two quarters of the atrium, and, as stated, becoming in its lower portion continuous with the second ganglion of the inter-atrial septum. (*c*) A small ganglion on the left wall of the left atrium about its middle.

The nerve cells in the cardiac ganglia of *C. auratus* are all of one type. This is of interest following the differentiation of ganglion cells into two types by Kiss (1932) in cranial and spinal ganglia, and by Bacsich (1933) in the cardiac ganglia of the pig: both of these authors used the method of prolonged osmic acid staining. Blair & Davies (1933, 1935) corroborated these findings using their pyridine-silver technique on human and bovine material. The two cell types of these authors are, first, a pale cell of round shape and finely stippled texture, and secondly, a smaller cell of rather angular form which stains almost black. The single cell type met with in the cardiac ganglia of this rodent is a large and round one with a fairly coarse granular cytoplasm. Its main characters would stamp it as belonging to the large pale type of ganglion cell, although it is not quite typical, being rather darker in general appearance and more coarsely granular (Pl. 2, fig. 11). Numerous nerve fibres could be traced into the substance of the atrio-ventricular node, particularly the meshwork portion, but only one

type of nerve ending could be identified, a simple filament ending applied to the muscle fibre. In contrast, the sinu-atrial node has a poor nerve supply, no fibres or cells being demonstrable in the substance of the node. This is quite different from the condition found in the rat. In that animal (Meiklejohn, 1913*b*), nerve fibres pass into the sinu-atrial node where they break up to form fine plexuses round the muscle fibres. The atrio-ventricular bundle is plentifully supplied with nerve fibres forming a well-marked neuro-muscular bundle. Again, only a filament type of nerve ending could be identified, no endings of the nature of sole-plates such as described in other animals by previous authors being found. It was noted, while tracing the limbs of the bundle, that, for some distance down the right side of the ventricular septum beyond the end of the right limb, its former position could be seen by the persistence of several well-marked strands of nerve fibres. No nerve cells occur in the atrio-ventricular bundle as described in *Cavia* (Meiklejohn, 1913*a*).

DISCUSSION

There are several features of interest in the specialized conducting tissue of this heart. The first of these is the occurrence of Purkinje fibres in the sinu-atrial node. Hitherto it has been held that such fibres occur in those parts of the specialized heart muscle, e.g. in the bundle of His and its limbs, where their characteristic structure is suited to the function of rapid conduction of the cardiac impulse. Physiological support for this belief is strong. Figures given by Lewis (1925) indicate a rate of conduction in Purkinje fibres four times as great as in the next most rapidly conducting part of the heart muscle. Of course, Purkinje fibres have been described as occurring elsewhere than in the atrio-ventricular bundle and its ramifications, but in such situations, for example in the subendocardial atrial network, their function is commonly presumed to be simply that of carrying on the contraction impulse. The demonstration of their presence actually in the sinu-atrial node, the pacemaker of the heart, adds support to the conception that a function other than conduction may be assigned to them. It may be, however, that their occurrence in the substance of the node is simply to ensure an immediate transmission of the cardiac impulse to the surrounding atrial muscle. The absence of Purkinje tissue in the atria, apart from the sinu-atrial node, in the rapidly beating hamster heart, is in striking contrast with the condition found in the avian heart. In the bird, in which the heart rate may be considerably greater than in the hamster, there is a well-marked subendocardial atrial network of Purkinje fibres (Davies, 1930).

The second point of interest is the compound nature of the atrio-ventricular node, part being composed of the typical meshwork arrangement of fibres and part of compactly arranged fibres, many of which approach in size and general appearance to the Purkinje type.

The last feature of special importance is the limited distribution of Purkinje tissue within the ventricles. Only in the left ventricle could any be found, and this at some distance below the point at which the left limb of the atrio-ventricular bundle becomes indistinguishable from the ordinary myocardium, continuity being established through structurally unspecialized ventricular muscle. In the right ventricle no Purkinje tissue whatever is present. A point of considerable interest is the unusual position of the Purkinje tissue in the left ventricle, being for the most part deeply placed within the thickness of the wall. This is in association with the very great thickness of the left

ventricle wall which can be more effectively brought into relation with the conducting tissue by this arrangement than by the more usual subendocardial spread. It is again important to compare the distribution of Purkinje tissue in the ventricles of the hamster with that of the bird. In the bird heart there is a subendocardial Purkinje network in both ventricles, and from this network Purkinje fibres penetrate through the thickness of the septal and free walls of the ventricles around branches of the coronary arteries, following in their course the pattern of the myocardial branches of the arteries, and reaching in some places to the subepicardial connective tissue (Davies, 1930). In each case, therefore, hamster and bird, the rapidly beating heart requires an intramural ventricular distribution of Purkinje tissue.

It is becoming increasingly apparent, as a knowledge of the comparative anatomy of the conducting tissue of the heart is acquired, that the typical bloated Purkinje fibre is not the sole criterion of conducting tissue; in the human heart such fibres may be absent from considerable parts of the atrio-ventricular bundle (Blair & Davies, 1935). These authors stress the importance of continuity with an established part of the conducting system as the criterion of this or that fibre belonging to part of the conducting system. The study of this rodent demonstrates the wisdom of this recommendation because here is a heart with no Purkinje tissue in the right ventricle and only a limited amount in the left (beyond the very abbreviated branches of the atrio-ventricular bundle), but which nevertheless maintains a cardiac rate of 450 cycles per minute.

Finally, mention must be made of a paper published recently by Glomset & Glomset (1940*a*) in which the existence of the sinu-atrial node is denied. These authors reached this conclusion after detailed study, macroscopic and microscopic, of numerous hearts from several different orders of mammals, viz. man, dog, ox, sheep, pig and horse.

SUMMARY

1. The specialized conducting tissue in the heart of the golden hamster is described.

2. Attention is drawn to the occurrence of large typical Purkinje fibres in the sinu-atrial node which is itself of considerable size.

3. The atrio-ventricular node and sinu-atrial node are not connected directly by any strand of specialized tissue.

4. Purkinje tissue in the ventricles is confined to the left ventricle whose wall is, over most of its extent, six times as thick as that of the right.

5. Nerve elements in relation to the specialized tissue are described.

I wish to express my gratitude to Prof. Blair for guidance and criticism, and also to Prof. Davies, of Sheffield, who has examined my slides and on many points given much helpful advice. To Prof. Hindle, of the Zoology Department, I am greatly indebted for the animals used in this work, and to Dr Knox, of the Physiology Department, for his services in taking the electrocardiograms.

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EXPLANATION OF PLATES 1 AND 2

All figures are untouched photomicrographs.

All the sections photographed were prepared by silver impregnation except those from which Figs. 1, 3 and 7 were taken. Figs. 1 and 3 are of sections stained with haematoxylin and eosin, and Fig. 7 is of a section stained Van Gieson.

Plate 1

Fig. 1. Transverse section of upper end right atrium near opening of superior vena cava. Note compact appearance of sinu-atrial node which occupies entire thickness of heart wall. ×40.

Fig. 2. Sinu-atrial node. Shows numerous Purkinje fibres in the substance of the node. ×560.

Fig. 3. Transverse section of atria. On right side of atrial septum is the atrio-ventricular node. ×24.

Fig. 4. Atrio-ventricular node. ×144.

Plate 2

Fig. 5. Transverse section through all four chambers to show atrio-ventricular bundle in the trigonum fibrosum dextrum, and right limb passing subendocardially down ventricular septum. ×24.

Fig. 6. Transverse section of aortic root. The upper slit-like part of the anomalous aortic recess is shown; note its proximity to the atrio-ventricular node. Compare with Text-fig. 5. ×32.

Fig. 7. The atrio-ventricular bundle is here seen straddling the ventricular septum. ×96.

Fig. 8. Coronal section of heart to show atrio-ventricular bundle cut in transverse section immediately prior to dividing into right and left limbs. ×144.

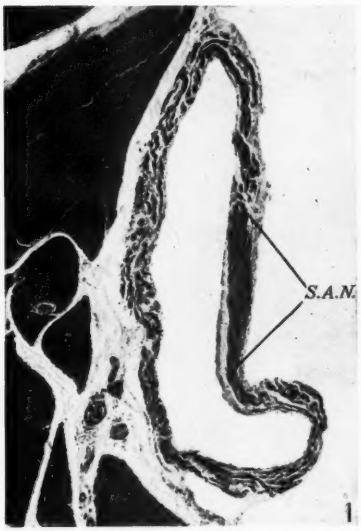
Fig. 9. Transverse section of left ventricle showing ventral plate of Purkinje fibres surrounded by a space. ×96.

Fig. 10. Purkinje fibres of left ventricle. ×480.

Fig. 11. Ganglion of atrial septum. ×560.

ABBREVIATIONS USED IN FIGURES

S.A.N. sinu-atrial node
A.V.N. atrio-ventricular node
A.V.B. atrio-ventricular bundle
R.A. right atrium
L.A. left atrium
L.V. left ventricle
R.V. right ventricle
Ao. aorta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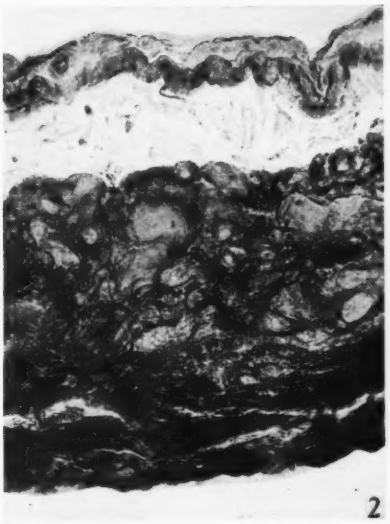
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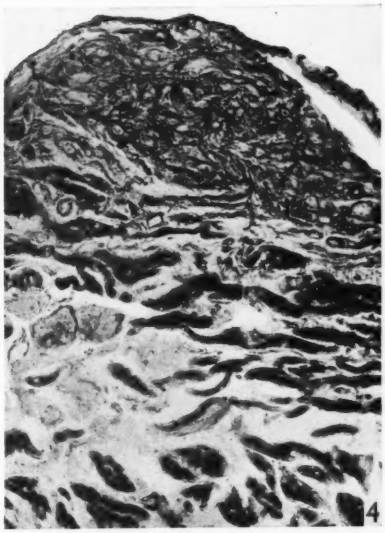
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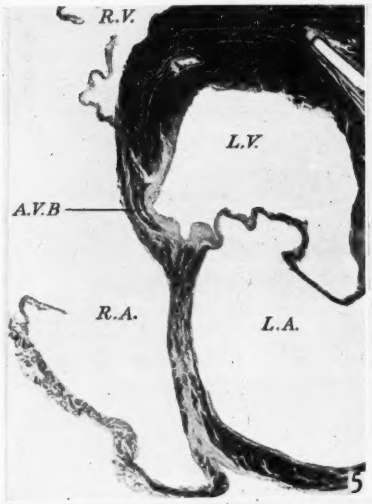
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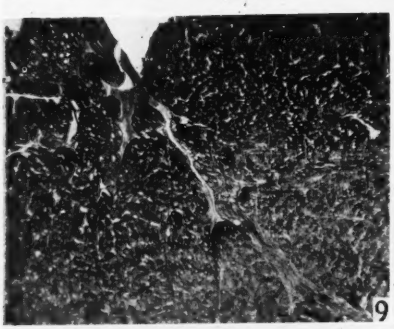
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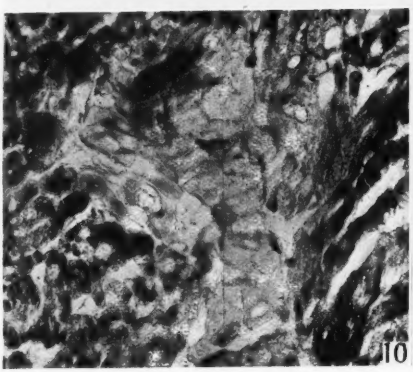
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THE CELLS OF MEYNERT IN THE VISUAL CORTEX OF THE MONKEY

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In 1872, Meynert called attention to conspicuous large cells which are present in the visual cortex of the human brain and which are characterized by possessing well-marked horizontally directed basal processes. He noted that cells of this type are located at the level of the white line of Gennari, and also in the infragranular layers of the cortex. The former are not so large or conspicuous as the latter, though in the visual cortex of some species of New World monkeys they may be aggregated to form a definite lamina traversing the central zone of the white line of Gennari. Those in the infragranular layers, however, are particularly obtrusive in stained sections, and since it is to these that the reference 'solitary cells of Meynert' is now commonly applied, such a usage of the term may conveniently be followed here.

The first detailed account of the morphology of the solitary cells of Meynert was provided by Cajal (1899). He described them as being situated in his 7th layer of the visual cortex of the human brain (a layer which evidently corresponds to at least a part of the lamina ganglionaris of more modern terminology) and he noted that they are commonly disposed in a single row, and at some distance from each other, in a plexus of fibres which are mainly horizontal. The cells were described as being characterized by having a base which is very broad in proportion to its height, and in possessing stout basal dendrites which extend for considerable distances parallel to the surface.

In many lower mammals the cells of Meynert seem hardly to be present in the visual cortex; at least, there are no cells in the infragranular layers which stand out conspicuously by reason of their size, shape and their basal dendritic expansions. Thus, in a recent intensive study of the cyto-architecture of the visual cortex of the rabbit, based on Golgi preparations, O'Leary & Bishop (1938) make no record of cells of exceptional size and with extensive horizontal dendrites in the infragranular layers. In some reports of the cortical cyto-architecture of lower mammals, based on Nissl preparations, references have from time to time been made to the presence of cells of Meynert, but it is not always certain that they can be correctly identified in such material. However, an exception occurs in the primitive mammal, *Tupaia*, for in the visual cortex of this genus scattered cells of unusually large size are very conspicuous elements in the deeper laminae of the visual cortex (Clark, 1925). The significance of this may be related to the concurrent development in *Tupaia* of an advanced degree of differentiation of the visual cortex with remarkably massive anterior colliculi in the mid-brain, for, as will be shown, there is evidence that these cells provide cortico-mesencephalic fibres.

OBSERVATIONS

Histological. In silver-impregnated sections of the visual cortex of *Macaca* Meynert cells are very conspicuous. In both pyridin-silver and Bodian preparations they become deeply stained, while the cytoplasm of other cells remains unstained or only slightly impregnated. The significance of the differential staining is not clear, and it

may be related simply to cell size. In any case, however, it permits of a ready recognition of the Meynert cells so that their distribution and enumeration can be easily determined. In methylene blue preparations they are also evident and are seen to contain well-defined Nissl granules scattered throughout the cytoplasm, but they do not stand out so conspicuously from other, smaller cells of the infragranular layers. In the present study, use was made of a series of Golgi preparations of the macaque visual cortex, in order to observe the relation of the cells of Meynert and their processes to other cells of the cortex. In Text-fig. 1 is a composite drawing made from these preparations. Each cell in the drawing has been accurately drawn from an individual cell in one of the sections and placed in its correct position relative to the laminar arrangement.

In general, the Meynert cells are situated in the lamina multiformis (lamina VI). Occasionally they are to be seen in the boundary between lamina V and lamina VI, and less commonly in lamina V. A similar stratigraphical location has been noted in *Papio* by Schuster (1910) and in *Cebus* by v. Bonin (1938). It appears, therefore, that in the monkey their level in respect of the cortical layers is not quite the same as in man, for according to Cajal (as noted above) and also according to the recent studies of Conel (1939), in the human brain they are characteristically found in lamina V. Measurements of fifty Meynert cells selected at random in silver preparations, made with an ocular micrometer, show that their size varies from $24 \times 14\,\mu$ to $43 \times 24\,\mu$, with an average of $29 \times 20\,\mu$ (the long axis being vertical). They are all cells of the pyramidal type, and, as in the human brain, they are characterized by an unusually broad base and by the great extent of the horizontal basal dendrites.

The apical dendrite, a remarkably stout process, runs vertically upwards to reach the zonal lamina at the surface of the cortex. It thus extends through almost the whole thickness of the visual cortex which (on the basis of measurements made on sections of twenty different specimens) has been found to have an average depth of 1·3 mm. Its course is straight except for slight sinuosities, and it usually forms a component of one of the radial fasciculi which are so well defined in the visual cortex. Its coarseness serves to distinguish it from the fine fibres of which these fasciculi are mainly composed. No branches are given off by the apical dendrite above the level of lamina V. At its origin, however, lateral branches are frequently given off which pursue a horizontal course in lamina V or VI, or an oblique upward course which is usually confined to lamina V. Occasionally in lamina V one or two collaterals arise which first run obliquely upwards through the upper level of lamina V and the lower level of lamina IV*c*, and then turn vertically upward in one of the radial fasciculi. These collaterals can be traced as far as the stria of Gennari (lamina IV*b*).

Of the basal dendrites of the Meynert cells, those which proceed horizontally are the most conspicuous. They confine themselves to lamina VI and the deeper levels of lamina V. In several instances they could be traced for a course of over 0·25 mm., and in the case of one cell the main horizontal dendrite on one side was followed under the microscope for a distance of 0·37 mm., and even then its actual termination was not identified. Occasional short branches arise from the proximal part of the main basal dendrite, but they limit their distribution to lamina VI. Other quite short dendrites leave the basal surface of the Meynert cell and extend downwards for short distances. From one of these the axone may arise, or it may take origin directly from the cell-body (Pl. 1, figs. 4, 5).

Text-fig. 1. Composite drawing of a section of the visual cortex of a macaque monkey, made from Golgi material. Two Meynert cells are represented in the lamina multiformis. The approximate extents of the cell laminae are indicated on the left. The axonal processes of the cells are marked *a*.

The Meynert cells are usually single, but occasionally two or three are found together. Counts were made in silver sections of the visual cortex in order to compute the number of Meynert cells in a given area of cortex. It was found that they are distinctly more numerous on the lateral surface of the occipital lobe than in the neighbourhood of the calcarine sulcus on the medial surface. In other words, the density of their distribution is greater in that part of the visual cortex which is concerned with macular vision. The figures obtained in these counts are as follows.

Lateral surface of occipital lobe. In a number of sections representing altogether a total linear cortical measurement of 161 mm., 184 Meynert cells were counted. Thus the average distance between adjacent cells is 0·86 mm. This corresponds to a density of approximately 130 cells in each square cm. of cortex.

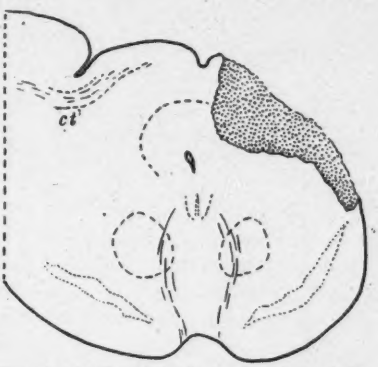
Medial surface of occipital lobe. In a number of sections representing altogether a total linear cortical measurement of 77 mm., 58 Meynert cells were counted. Thus the average distance between adjacent cells is 1·33 mm. This corresponds to a density of approximately 56 cells in each square cm. of cortex.

In order to compute the total number of Meynert cells in the visual cortex of the macaque monkey, it was necessary to estimate the total surface area of the visual cortex. For this purpose, a male animal (O.M. 212) of average size was selected. The third molar was in process of eruption, the body weight was 3980 g., and the brain weight 88 g. The brain was fixed in Kaiserling solution. Both occipital lobes were cut coronally in sections of 1 mm. thickness with a freezing microtome. The sections were then placed in glycerin. By transmitted light the white line of Gennari was clearly visible as a dark band, and allowed the limits of the visual cortex to be defined with accuracy. In each section the surface contour of the visual cortex was traced on to cellophane with indian ink. The length of each contour was subsequently measured with fine thread. With these data the total area of the visual cortex could be calculated. On the left side the area was computed to be 1462 mm.^{2} and on the right side 1428 mm.^{2} The average area of the visual cortex on each side may therefore be taken to be approximately 1450 mm.^{2} Further analysis of the measurements showed that approximately 46 % of the whole extent of the visual cortex is accommodated on the lateral surface of the occipital lobe, the remaining 54 % being confined to the medial surface and mainly buried within the lips of the calcarine sulcus. From these data it may be estimated that, in the brain of an average-sized monkey, the total number of Meynert cells in the whole visual cortex of either hemisphere is of the order of 1300.

Experimental. The functional connexions of the large solitary cells of Meynert have not been established. It has been generally assumed that they are concerned with the efferent functions of the visual cortex, mainly on account of their size, and also their position in the infragranular cell laminae, for the comparison which they invite with the large Betz cells of the motor cortex is obvious. In a study of the isolated visual cortex (Clark & Sunderland, 1939) the atrophy of the Meynert cells was found to be one of the most conspicuous results of severing all the connexions of a small area of cortex, and since they were unaffected when only the connexions with adjacent areas of the cortex are interrupted, or when the whole of the parietal cortex is removed (Clark & Northfield, 1937), it was inferred that they give rise to efferent projection fibres passing to subcortical centres, possibly the region of the tectum of the mid-brain. In order to test this inference it was decided to interrupt the cortico-mesencephalic fibres by an operative lesion on one side of the brain in a rhesus monkey (O.M. 504)

with the object of seeing whether the Meynert cells were affected by a retrograde degeneration. Previous experience had shown that it may be by no means easy to differentiate cells undergoing chromatolysis after axonal interruption from the cytological variations of normal nerve cells; hence we did not approach the experiment with the expectation that it would give a definite answer to our question. Moreover, it is well known that if, at its origin from the cell body, a number of short collaterals are given off by the main axone, section of the latter may not lead to easily recognizable retrograde cell atrophy, for the effect of the lesion is simply to convert a long-axone cell into a short-axone cell. However, the results of the experiment seem to be sufficiently conspicuous to permit of positive conclusions.

At the operation a large osteoplastic flap was turned down on the left side, exposing the whole of the parietal and occipital lobe. The occipital lobe was mobilized by coagulating and severing the cortical veins which terminate in the sagittal and lateral sinuses, and it was lifted up to expose the tentorium cerebelli. The latter was then incised, and by reaching carefully forward the superior colliculi were brought to view. Using the diathermy current, a lesion was made along the lateral margin of the left colliculus in order to interrupt the cortico-tectal fibres which enter the mid-brain in this region.



Text-fig. 2. Drawing of a section through the mid-brain of monkey O.M. 504, showing the extent of the lesion in the left superior colliculus. The position of the cortico-tectal tract is shown on the right side (*ct*).

The animal made a good recovery, but since it later developed signs of cerebral irritation it was killed a week after the operation, earlier than had been intended. On exposure of the brain it was found that the involvement of cortical vessels had led to some necrosis of the cortex on the medial side of the occipital lobe. The remainder of the cortex, however, was intact and the lesion in the mid-brain proved to be very satisfactorily placed (Text-fig. 2), destroying the major part of the left superior colliculus and certainly interrupting all the cortico-tectal fibres.

Longitudinal sections were taken through the occipital lobes of both sides, and stained with gallocyanin. A study of these sections provided definite evidence of early degenerative changes in the large cells of Meynert on the operated side. On the unoperated side these cells show the normal appearance of a large pyramidal or polygonal cell, with a central nucleus, and with abundant fine Nissl granules scattered evenly through the cytoplasm (Pl. 1, fig. 1). On the operated side, some apparently normal cells were found, though in these the Nissl granules appear hyperchromatic. In most of the cells, however, signs of incipient dissolution are evident, such as the excentricity of the nucleus (which in some cases bulges up to the surface of the cell), vacuolation of the cytoplasm, and dispersal of the Nissl substance (Pl. 1, fig. 2). In some cells the vacuolation has led to the formation of vesicular protrusions (Pl. 1, fig. 3). The Nissl substance is characteristically pressed to the periphery of the cell body, or to the margins of the vacuolations where these are present.

There is no doubt from these experiments that many of the Meynert cells on the

operated side show the early reactions characteristic of axonal interruption. By itself the experiment does not exclude the possibility that this is related to the involvement of the visual cortex on the medial aspect of the occipital lobe, and that the cells may therefore give rise to intrastriate association fibres. This interpretation, however, is definitely negatived by previous studies (Clark & Sunderland, 1939; Clark, 1941) which led to the conclusion (1) that the Meynert cells give rise to subcortical efferent fibres of some kind, and (2) that in any case the intrastriate association fibres of the visual cortex do not extend from any point for more than a few millimetres. It may therefore be accepted that the reaction shown by the Meynert cells in the present experiment is a retrograde degeneration following on the interruption of the cortico-mesencephalic fibres from the visual cortex where they enter the mid-brain. This does not, of course, exclude the possibility that other cellular elements of the visual cortex may also contribute cortico-mesencephalic connexions. With their smaller size and less distinctive Nissl pattern, however, it is hardly possible to recognize with certainty retrograde degenerative changes in them so early as the seventh day.

DISCUSSION

The histological details of the Meynert cells of the visual cortex in the macaque brain and the experimental evidence bearing on their distant connexions allow certain fairly definite conclusions to be drawn regarding their functional significance. The total number of Meynert cells in the visual cortex is relatively small. On the other hand, their basal dendrites spread out tangentially to the surface in all directions for considerable distances. It has been recorded that in one instance a basal dendrite was traced for a distance of 0·37 mm. without terminating. It thus appears that by the basal dendrites each cell may 'drain' a surface area of cortex approximating to $\frac{1}{2}$ mm.^{2} (and perhaps more). The apical dendrite receives impulses directly from the zonal lamina, and by its collateral branches, from laminae IV*b*, IV*c* and V. On the other hand the cell seems not to be directly connected by its dendrites with the pyramidal layer (lamina III) or lamina IV*a*. It may be inferred, therefore, that the Meynert cells are activated predominantly by neuronal activities occurring in deeper layers of the cortex (particularly lamina VI) and by those which occur in the lamina zonalis. The latter, it should be noted, almost certainly reflects in some degree the activities of all the subjacent cortical laminae, since it receives impulses from many cells with ascending axones, as well as the terminals of the numerous ascending collaterals which take origin from the descending axones of typical pyramidal cells. Each Meynert cell is thus ultimately brought under the influence of all the layers of the cortex within its particular zone. It is a focus of convergence for the activities of these layers and its axone represents a 'final common path' for a unit area of the visual cortex of approximately $\frac{1}{2}$ mm.^{2} The experimental evidence indicates that the axone of each Meynert cell serves to convey impulses from each such unit area directly to the mid-brain—whether to the tectum or to adjacent mesencephalic regions is not certain. Although the existence of cortico-mesencephalic connexions from the occipital lobe is well established, there has been some uncertainty regarding their precise origin. Poliak (1932) was inclined to believe, on the basis of Marchi experiments on monkeys, that they are derived from the peristriate cortex rather than the striate cortex proper. On the other hand, the more recent observations of Walker & Weaver (1940), that it

is possible to elicit contralateral conjugate deviation of the eyes of the monkey by stimulation of the cortex of the area striata, appear to provide conclusive evidence that at least some cortico-mesencephalic connexions arise from this area. The probability should be noted that other elements of the visual cortex besides the large cells of Meynert to which this paper refers may also give rise to these connexions. Particularly may this be the case with the large cells in lamina IV*b*, which Meynert himself included among the conspicuous cell types of the visual cortex to which he originally drew attention. These cells in lamina IV*b* have also been observed to undergo marked atrophy in isolated portions of the cortex (Clark & Sunderland, 1939). The functional significance of the cortico-mesencephalic connexions has been indicated in a discussion by Holmes (1938) on the cerebral integration of ocular movements. Referring to the occipital oculomotor centre, he concludes that it is concerned with the reactions necessary for the unification of separate impressions arising from the two retinae, with involuntary movements of the eyes towards objects in the peripheral field of vision, and with the maintenance of fixation on a point whether the latter is stationary or in movement.

The author's thanks are due to Mr E. Thompson for his technical skill in the preparation of the histological material, and to Mr W. Chesterman for the excellent photomicrographs.

SUMMARY

1. Solitary cells of Meynert are very conspicuous elements in the visual cortex of the macaque monkey. They are situated mainly in lamina VI and are characterized by the great extent of their basal dendrites.

2. It is estimated that there are approximately 1300 Meynert cells in the area striata of each hemisphere in *Macaca*. They occur at an average interval of 0·86 mm. on the lateral surface of the occipital lobe and of 1·33 mm. on the medial surface.

3. Experimental evidence is adduced in favour of the conclusion that the axones of the Meynert cells contribute to the cortico-mesencephalic connexions.

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EXPLANATION OF PLATE 1

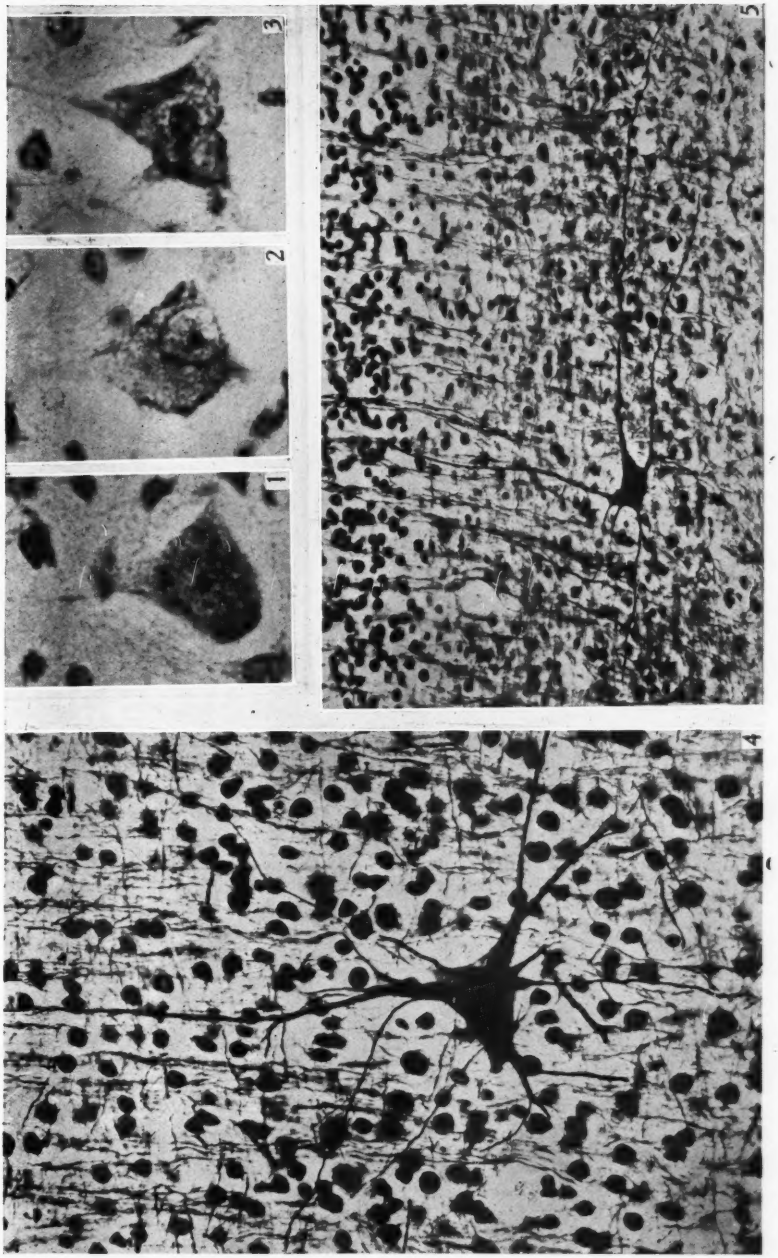
Fig. 1. Meynert cell in normal visual cortex. Note the central position of the nucleus and the even distribution of Nissl granules throughout the cytoplasm. Gallocyanin. ×800.

Fig. 2. Meynert cell from operated side in experiment O.M. 504. Note the excentricity of the nucleus and the peripheral displacement of the Nissl substance. Gallocyanin. ×800.

Fig. 3. Meynert cell from the operated side in experiment O.M. 504. Note the large vacuolation at the left side of the base of the cell. Gallocyanin. ×800.

Fig. 4. Normal Meynert cell in the visual cortex of a macaque monkey. The apical dendrite can be seen extending vertically towards the surface of the cortex. From the middle of the basal aspect of the cell the axone descends vertically towards the white matter. Bodian's protargol technique. ×470.

Fig. 5. Normal Meynert cell, showing the great horizontal extent of the basal dendrites. Bodian's protargol technique. ×300.



Figs. 1–5.

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THE EARLY DEVELOPMENT OF THE GOAT (*CAPRA HIRCUS*)

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INTRODUCTION

Despite the vast amount of literature and study devoted to early mammalian development, there are few available references which deal with the early cleavage stages in the goat. So far as we can ascertain, only two observations have been made on the living goat egg; one by Krause (1837) who measured three ripe ovarian eggs, using a measurement open to doubt, but from which Hartman (1929) estimated the size of the living goat egg to be of the order of 140 μ, a figure which is remarkably close to the average diameter of 145·3 μ for the ova measured in the present investigation; the other by Amoroso, Griffiths & Hamilton (1939*b*) who described and figured tubal eggs in the one-cell, two-cell and four-cell stages. Assheton's (1908) youngest of five specimens (copulation age 5 days 20 hr.) had already left the tube and is at a stage in development comparable to the oldest of the uterine eggs we describe. The only other contribution is that of Tsukaguchi (1912), who described several stages of the goat's blastocyst (age not stated), the earliest of which corresponds closely to Assheton's specimen of the sixth day. Like Assheton, Tsukaguchi does not describe or figure the segmenting stages. The present contribution attempts to complete the lacunae in our knowledge in both these respects.

MATERIAL AND METHODS

The material for that part of the investigation which is reported in this paper was obtained from a group of fifteen females, during the breeding seasons from September 1937 to January 1940. Since the mutual behaviour of the nanny and billy alone constitutes a reliable indication of oestrus, the most satisfactory criterion for its detection was the introduction of the male among the females at frequent intervals (from 4 to 6 hr.), and observing the willingness of the females to associate with the males. When a particular female was found to associate freely with the male she was removed from the flock and suitable precautions were taken to prevent mating until it was decided to breed from her. Oestrus was dated from the time the female would 'stand' and allow the male to mount to the time of last acceptance of the male. Since ovulation is said to occur towards the end of heat, the animals were bred as late in heat as was possible and killed at the times necessary for recovering eggs of the desired ages.

In securing the eggs the methods used were essentially those devised by Corner (1917) for obtaining pig eggs and adequately described by Clark (1934) for the sheep. The method employed is satisfactory, since in the great majority of cases the number of eggs recovered always corresponded with the number of freshly ruptured follicles. After recovery most of the eggs were photographed while fresh in isotonic saline and

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some after fixation. The intact eggs were photographed with a Leitz 'Makam' attachment, fitted in some cases with a Leitz 'Ultropak' illuminator (see Pl. 1, figs. 1, 5 and 9). Most photographs were taken at a magnification of ×95 and then enlarged to ×250. A few were, however, taken at ×200. All measurements were made from photomicrographs of known magnification.

The fixatives used were the solutions of Bouin, Flemming and Zenker, the latter with a little acetic acid. These were found satisfactory. Various procedures were tried for embedding the eggs, since it was essential to have a method sufficiently reliable to deal with the limited amount of material available. The method finally adopted was a modification of the method devised by Hill & Tribe (1924) for dealing with cat eggs. It will be described elsewhere. Serial sections 5 μ thick were cut and stained with Heidenhain's iron haematoxylin or with Weigert's haematoxylin followed by eosin.

In order to determine more accurately the spatial relationships of the blastomeres and to determine their volumes, a series of dissectable wax reconstruction models at a magnification of ×800 were made. These are illustrated in Text-figs. 1–4.

CLEAVAGE STAGES

One-cell stage: first segmentation spindle

Two unsegmented eggs (14A and 4B) are represented in our material. One of these, 4B (Pl. 1, figs. 1, 2), is at the stage of the first segmentation spindle and was recovered with its sibling, a two-cell egg, approximately 30½ hr. after insemination. The other egg, 14A (Pl. 1, fig. 3), was washed from the left uterine horn together with another egg, more advanced in development, precisely 140 hr. after mating. It is perhaps a degenerate or pathological egg which would not develop. These eggs are almost if not quite spherical, and the diameters of the two, as measured on the photographs, are given in Tables 1 and 2. As is apparent from Table 2, the difference in the volumes of the vitelli of these two eggs is considerable. Egg 14A, having a post-coital age of 140 hr., has not developed to schedule, since normal eggs would be in the morula or early blastocyst stages.

The zona pellucida of egg 4B (Pl. 1, figs. 1, 2) has an inner diameter of 145 μ, an outer of 170 μ, and a thickness of 12·5 μ. It appears quite homogeneous and is completely devoid of cumulus cells, although a number of spermatozoa can be seen embedded in it. The nearly spherical vitellus almost completely fills its cavity, leaving, however, an evident perivitelline space which, at this stage, is free of any granular detritus. A polar body was not seen in the living egg but was plainly visible in the sectioned material. In the morphology of the vitellus itself the most conspicuous feature is the presence of fat granules of various sizes in its cytoplasm. These are embedded in a somewhat dense mottled matrix, and can be best seen in the central region of the egg, where they are sufficiently concentrated to obscure the nucleus. The peripheral zone is clearer and has fewer granules; here they form a marginal ring immediately beneath the vitelline membrane and there is no trace of the polar accumulation of fat as described in some mammalian eggs.

The egg shown in Pl. 1, fig. 3, is quite different in appearance and is illustrative of the fate of the unfertilized egg. The yolk spheres are larger and no longer discrete, being clumped towards the centre of the cell, thereby increasing the opacity in this

region. The vitellus is shrunken, its volume having shrunk from 1,204,260 to 974,348 μ^3, almost 20%. The shrinkage is, however, uniform, since the egg retains its almost spherical outline. In the sectioned egg (Pl. 2, fig. 24), the lacunae of the fat globules in the peripheral zone are larger than in the fertile egg and their distribution is patchy, as in degeneration.

The sectional series of the fertile egg 4B (Pl. 2, figs. 22, 23) reveals that this egg is in the phase of the first cleavage spindle, a stage which is reached just under 31 hr. after insemination. The cytoplasm of this egg, which is everywhere in contact with

Table 1. *Diameters and volumes of zonas in microns and cubic microns*

Stage of eggs. *Mod.*: mean outer diameter of zona. *Mid.*: mean inner diameter of zona. *Z.P.*: thickness of zona. *Vol.*: corresponding volume.

Eggs	Stage	Mod.	Mid.	ZP.	Vol.
14 A	1-cell	164	143	10·5	1,531,112
4 B	1-cell	173	145	14·0	1,596,256
4 A	2-cell	174	145	14·0	1,596,256
1 A	2-cell	175·3	152	11·65	1,838,778
5 A	4-cell	176·6	156·6	10·0	2,007,035
5 B	4-cell	163·5	145·0	9·25	1,596,256
2 A	8-cell	178·3	152·6	12·8	1,855,043
2 B	8-cell	178·3	152·6	12·8	1,855,043
14 B	9-cell	168·0	142·0	13·0	1,499,214
12 A	12-cell	168·3	143·3	12·5	1,547,284
12 C	13-cell	172·0	147·0	12·5	1,663,224
7 A	32-cell	166·3	136·3	15·0	1,331,723
7 B	30±2-cell	167·6	138·0	14·8	1,376,055
7 C	30-cell	163·3	139·0	12·0	1,406,187
13 B	96-cell (EB)	168·3	143·3	12·5	1,547,284
8 A	126-cell (B)	170·0	145·0	12·5	1,596,256
	Average	170·4	145·3	12·5	1,615,188

Table 2. *Diameters and volumes of vitelli in microns*

Stage: stage in development of eggs. *SMD.*: mean diameter of smallest vitellus or cell. *LMD.*: mean diameter of largest vitellus or cell. *AMD.*: average mean diameter of vitelli or cells. *S.Vol.*: volume of smallest vitellus. *L.Vol.*: volume of largest vitellus. *A.Vol.*: average volume of vitelli.

Eggs	Stage	SMD.	LMD.	AMD.	S.Vol.	L.Vol.	A.Vol.
14 A	1-cell	—	—	123·3	—	—	974,348
4 B	1-cell	—	—	132·0	—	—	1,204,260
4 A	2-cell	105·3	123·3	117·0	606,131	974,348	838,603
1 A	2-cell	101·6	113·0	109·2	539,464	735,499	678,076
5 A	4-cell	76·6	92·0	86·8	234,414	407,721	338,882
5 B	4-cell	73·3	88·3	83·3	207,903	362,935	304,831
2 A	8-cell	66·6	73·3	70·8	153,980	207,903	183,471
2 B	8-cell	64·6	76·6	72·6	140,501	234,414	199,532
14 B	9-cell	48·3	73·3	64·9	59,734	207,903	143,794

the zona, consists of a narrow homogeneous and more deeply staining superficial zone which is thicker at the pole adjacent to the polar bodies than elsewhere, and a reticular, more lightly staining central mass in which the nucleus is embedded. The lacunae of the larger fat globules are not very abundant and are mainly grouped about the superficial zone. The nucleus which is in anaphase is practically centrally situated, and extends through three sections, two of which are shown in Pl. 2, figs. 22, 23. The mitotic figure consists of two clumped masses of chromosomes still united by spindle fibres, with remains of the asters in the vicinity of the chromosomes. As the chromosomes are not all at the same level, it is impossible to be sure of their number. The

two polar bodies which do not occur in the sections figured are adjacent to each other and lie in a distinct bay at the surface of the egg. Each possesses a single spherical mass of chromatin and they are so placed that a line joining their centre to the equatorial plate or midpoint of the spindle would cut the latter almost at right angles. It is suggested that division which is imminent would be at right angles to this line.

Two-cell stage

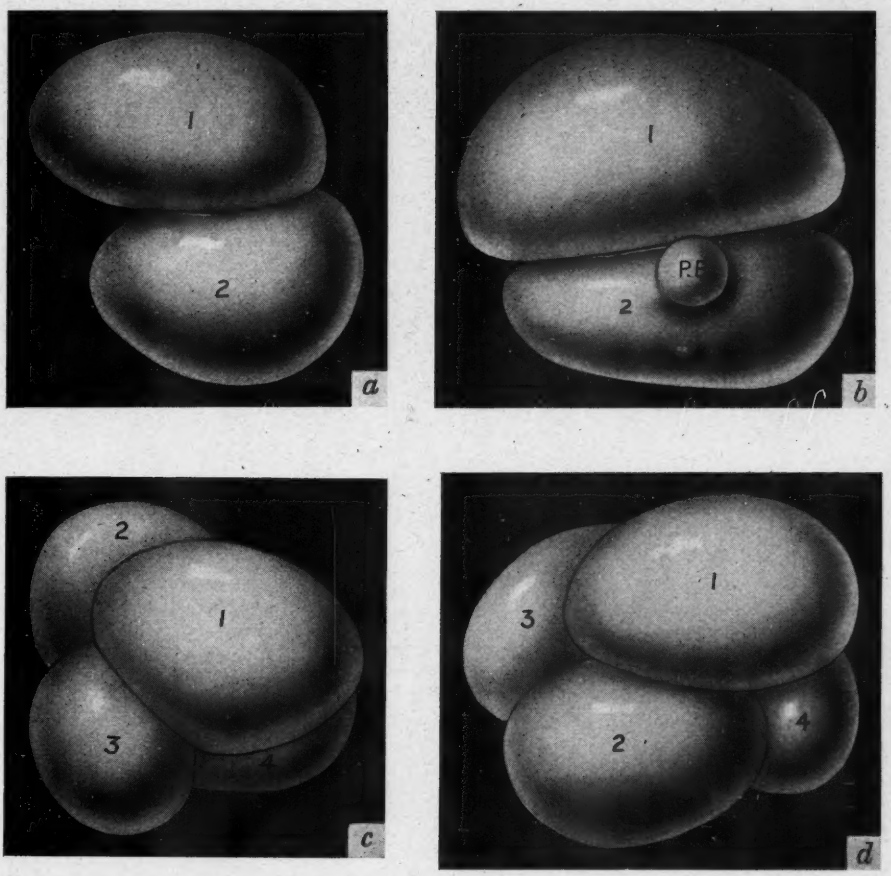
We have been fortunate in securing two tubal eggs, one (no. 4A) at the initiation, the other (no. 1A) at the completion of the two-cell stage. These eggs (Pl. 1, figs. 4–6) were recovered 30½ and 48 hr. after mating respectively, and in both the first cleavage is complete. Before discussing these eggs an interesting observation in one instance should be mentioned. The egg in Pl. 1, figs. 5, 6, was seen by one of us (W. F. B. G.) to complete its division from one to two cells in the interval which elapsed between recovery and photography.

The two blastomeres resulting from the first division are not of equal size, but they are both alike in structure in the photographs. Our measurements of the sizes of the two cells and the inner and outer diameters of the zonas are given in Tables 1 and 2. The vitelli have much the same appearance as in the uncleaved egg, differing from it only in size and shape. They are both ovoid and are in contact over a fairly wide area, with their longer diameters (Pl. 1, fig. 5) parallel to the cleavage plane and the shorter at right angles to it. The spatial relationship of the constituent cells is well illustrated in the models of these eggs (Text-fig. 1 *a*, *b*). In Pl. 1, fig. 6, where the cells measure 130×110 and $108 \times 100\,\mu$, the area of contact is $3817{\cdot}23\,\mu^2$, while in the slightly older egg (Pl. 1, fig. 4) with cells measuring 122×95 and $110 \times 85\,\mu$, it is $3028{\cdot}49\,\mu^2$. In the living egg there is no suggestion of polarity in the two-cell stage, the distribution of yolk granules being almost the same as in the late one-cell stage. The cytoplasm of each shows the same concentration of yolk material in the centre and the fat appears to be equally distributed between the two cells. The nucleus remains obscured and a polar body with a single chromatin mass lies on the surface of the smaller cell in Pl. 1, fig. 6. In this figure, the polar body measures $15 \times 10\,\mu$ and has a surface area of $561{\cdot}9\,\mu^2$ and a volume of $1252{\cdot}8\,\mu^3$. In the intact egg, it was not in contact with the zona and moved freely in the perivitelline space. The zona is free of cumulus cells although a number of spermatozoa are embedded in it.

The nuclei in the blastomeres of the early two-cell stage are illustrated on Pl. 3, figs. 25–27. Both are fully constituted and alike in structure, appearing as large, smooth, clear spheres almost in the centre of each cell. Each possesses one nucleolus and peripheral chromatin granules; both lie in the pale staining, finely reticular zone of the cytoplasm. In the larger cell, the peripheral accumulation of homogeneous cytoplasm stretches round the circumference of the egg without, however, invading the region below the contact surfaces. In the smaller blastomere it is restricted to one pole, so that a distinct polarity is present in this cell. Fat globules (small to medium) are present in abundance throughout the body of the egg. In Pl. 3, fig. 27, a polar body with a single chromatin mass and large fat vacuole lies in contact with the zona and smaller blastomere in the vicinity of the superficial groove between the two cells.

In our second egg of this stage the two blastomeres are again of unequal size (Pl. 1, fig. 4). Apart from this, there are no obvious structural differences between the two

cells, nor do the cells differ from those of the previous egg, except in regard to size and nuclear changes associated with their varying states of mitotic activity. The sectional series of this egg shows it to be more advanced in development. Both blastomeres (Pl. 3, figs. 28, 29) possess equatorial plates, with chromosomes clumped in metaphase. In the larger cell, that on the right in fig. 28, the mitotic figure has been



Text-fig. 1. Models of eggs nos. 4A and 1A (*a*, *b*), two-cell stages and nos. 5A and 5B (*c*, *d*), four-cell stages. ×600. *P.B.* polar body.

cut transversely, whilst in the smaller, that on the left in fig. 29, the plane of section is parallel to the axis of the spindle. Accordingly, the plane of division of the larger blastomere would be at right angles to the plane of the first cleavage, while that of the smaller cell would be parallel to it. With the completion of this division, then, the blastomeres of the four-cell stage should be grouped in two pairs, so arranged as to form a cross-shaped figure. As far as is known, these eggs constitute the first initial cleavage stages of the goat's ovum to be recorded.

Four-cell stage

We have available in our collection two eggs (5A, 5B) at this stage of development, which were recovered from the fallopian tubes of a female killed 60 hr. after copulation. In these eggs the conditions foreshadowed by the position of the cleavage planes of the two-cell stage are established, and the four blastomeres are readily grouped into pairs of very unequal size, so arranged as to form a rather irregular cross (Pl. 1, figs. 7–9 and Text-fig. 1 *c*, *d*). Of the two pairs of cells in each specimen, pair 1 and 2 occupy one hemispheres and 3 and 4 the other, the place of division between 1 and 2 in each case being almost at right angles to that between 3 and 4.

Though considerably smaller, the blastomeres repeat the structural design of the one- and two-cell vitellus, but both specimens are noteworthy in that each shows some inequality in the distribution of fat, one pair being richer in fat globules than the other. Many of these globules reach a much larger size than in previous stages, and are mainly located at opposite poles of the cells, though they extend up to the central region. The similarity in distribution of the fat, more especially in regard to

Table 3. *Relative volume of blastomeres calculated from wax-plate reconstructions*

The cells have been arranged in descending order of magnitude to emphasize the striking fact that there is invariably one small cell.

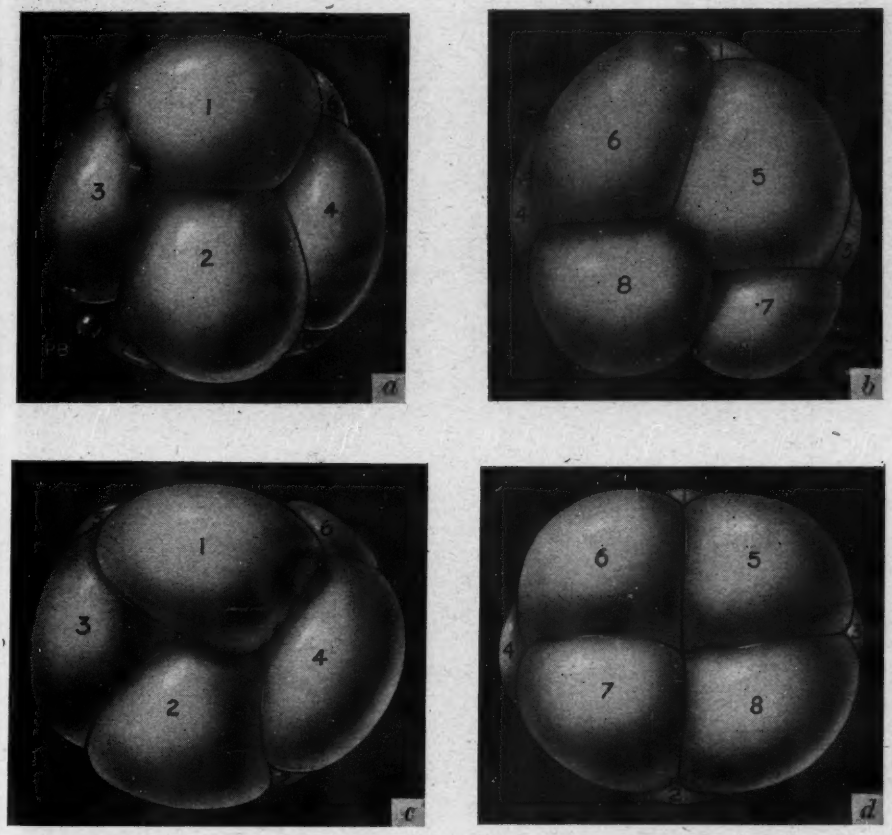
Cell stage	Egg	Percentage of total volume represented by each blastomere												
		A	B	C	D	E	F	G	H	I	J	K	L	M
2	4 A	57·89	42·10	—	—	—	—	—	—	—	—	—	—	—
	1 A	56·00	44·00	—	—	—	—	—	—	—	—	—	—	—
4	5 A	30·40	27·20	25·60	16·80	—	—	—	—	—	—	—	—	—
	5 B	29·60	27·60	26·30	16·40	—	—	—	—	—	—	—	—	—
8	2 B	16·17	12·74	12·25	12·25	12·25	12·25	12·25	9·80	—	—	—	—	—
	2 A	14·80	13·36	12·87	12·37	12·37	11·88	11·38	10·89	—	—	—	—	—
9	14 B	17·51	14·60	12·40	12·40	10·94	8·75	8·75	7·30	7·30	—	—	—	—
10	12 B	14·70	12·25	11·27	10·78	10·78	9·80	8·33	8·33	7·84	5·88	—	—	—
12	12 A	13·85	12·04	9·63	9·04	9·04	9·04	9·04	7·22	6·02	6·02	6·02	3·01	—
13	12 C	11·50	10·25	9·60	9·60	9·60	8·33	7·69	6·41	6·41	5·75	5·75	4·50	4·50

the occurrence of the larger globules, is strikingly illustrated in Pl. 1, figs. 7–9, a large globule appearing in one blastomere in each of the eggs. In the intact egg, a nucleus is visible in some of the cells. The zona is normal in appearance and a perivitelline space is present, but we did not observe any movement of the blastomeres. No polar body was visible.

In the sectioned material, the quantitative differences in the distribution of the fat globules and the structural condition of the nuclei in the two eggs are shown in Pl. 3, figs. 30, 31. Apart from the varying distribution of the fat globules and the differences in size of the cells, the only other structural changes are those associated with the varying states of division of the nuclei. In this latter respect, egg no. 5B, from the left tube, appears to be younger, since all its nuclei are in the resting phase, whilst in no. 5A from the opposite tube, the spindle of division has already appeared in the smallest cell (Pl. 3, fig. 30). In both eggs there is present in the interval between the two larger blastomeres remnants of the polar bodies, whilst between the zona and the superficial grooves between the cells, cytoplasmic detritus has accumulated.

The measurements of the volumes of the blastomeres of the four-celled eggs are summarized in Table 3 (5A and 5B). From these data, it is seen that the diversity

in cell size is more striking than at the two-cell stage. In this connexion it will be recalled that Heuser & Streeter (1929) found in the four-cell stage of the ovum of the pig differences in the size of the blastomeres which approximate very closely the figures given in Table 3.



Text-fig. 2. Two views of models of eggs no. 2B (*a*, *b*), and no. 2A (*c*, *d*), eight-cell stages. ×600. *P.B.* polar body.

Eight-cell stage

This stage is represented in our series by a litter of two eggs (nos. 2A and 2B) from the same tube, obtained 85 hr. after insemination. These two eggs, except for slight differences in their nuclear pattern, are essentially similar. The data recording the measurements of the living eggs are given in Table 1, and a photograph of one of them, no. 2B, is shown in Pl. 1, fig. 10. From these data and from the photograph it is manifest that the eggs have not altered appreciably in size, but the blastomeres are unquestionably smaller and more nearly spherical. In each egg the blastomeres are fairly closely adherent to one another and to the inner surface of the zona, where

they are somewhat flattened; between them they share fifteen large and two small contact surfaces. Two of the cells in each egg are in contact with five others, while the remainder adhere to four of their neighbours. There seems to be little variation in the arrangement of the cells in the two specimens, and they conform to the low-surface tension type described by Lewis & Wright (1935) for mouse eggs.

The eight blastomeres are arranged as shown in the figures of the models (Text-fig. 2 *a*, *b*, *c* and *d*), and the percentage of the total volume of each blastomere is given in Table 3 (2A and 2B). The arrangement of the cells as seen in the views of the models is surprisingly similar, and the measurements of the two eggs (Table 1, 2A and 2B) are actually identical. From the data in Table 3, it is seen that one cell appears to be constantly large (2 in the text-figure) and one small (7 in the text-figure), while the size of each of the other six cells lies somewhere between the two extremes. It will be noticed that these measurements for our eight-cell goat eggs approximate very closely those given by Gregory (1930) for the eight-cell rabbit egg.

In their sectional anatomy (Pl. 3, figs. 32–34) the cells retain many of the characteristics of the younger stages. The fat globules are as large as in the four-cell egg, and are as unevenly distributed, but the nuclei, hitherto smooth and membranate when at rest, have become lobed. This lobation is not shared equally by the two eggs, the nuclei of which are all at rest: those in egg 2A (figs. 32, 33) are all bilobed and in general have their chromatin peripherally arranged, while those in 2B (fig. 34) are more spherical and possess several central chromatin masses. In both these eggs, in the central grooves between the blastomeres, there is present a considerable amount of cytoplasmic detritus in which are small granular spherical masses.

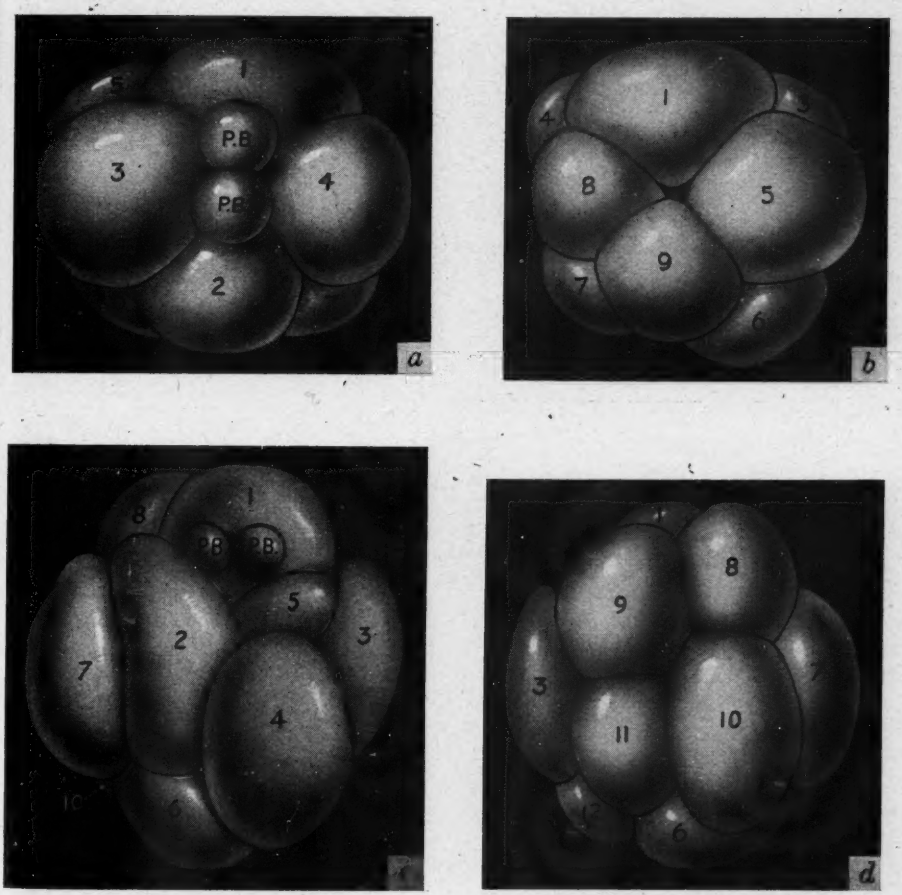
Nine-cell stage

There is in our collection a single nine-cell egg (no. 14B) which was obtained with its sibling (no. 14A), an unfertile egg, 140 hr. after insemination. This uterine egg, though belated in development, does not appear in the photograph of the fresh specimen (Pl. 1, fig. 11) to differ from the preceding stages in any recognizable respect except size and number of blastomeres. Notwithstanding its unpunctuality it is worthy of a brief description, since there are signs of unmistakable polarity in respect of the position of the constituent cells, which are segregated into groups of larger and smaller cells occupying opposite poles (Pl. 1, fig. 11 and Text-fig. 3 *a*, *b*). In the models the larger cells are numbered 1, 3, 4 and 5. The volumes of these cells were determined and the results are summarized in Table 3 (14B).

When the sectioned material (Pl. 3, figs. 35, 36) is examined the constituent cells are less compactly arranged than in previous stages and all contain numerous larger and smaller fat vacuoles distributed equally in each cell; also in each cell a small crescentic cytoplasmic area, which is non-reticular, is seen. Sometimes this area is at the sides of the cell directed towards the periphery and is in contact with the zona pellucida: at other times it is towards the centre of the egg (see fig. 35). In some of the cells are lobed nuclei, and in the superficial groove between the larger blastomeres and the zona there is present what appears to be two masses of cytoplasm (*P.B.* in the models) about half the size of the smallest blastomere, but non-nucleated and devoid of chromatin. Since we are not satisfied that the egg is quite normal, we do not venture to suggest what is the nature of these bodies—whether cell remains or cytoplasmic extrusion.

Ten-, twelve- and thirteen-cell stages

Female no. 12 provided the three uterine morulae (12B, 12A and 12C) which are available for the description of these stages. They were recovered from an animal killed 98 hr. after a single service, no. 12B with ten cells and no. 12C with thirteen coming from the left uterine horn, whilst no. 12A was obtained from the right horn.



Text-fig. 3. Two views of models of eggs no. 14B (*a*, *b*), nine-cell stage and no. 12A (*c*, *d*), twelve-cell stage. ×600. *P.B.*, *a*, non-nucleated cytoplasmic bodies; *P.B.*, *c*, polar bodies.

These three eggs differ from each other not only in the numbers but also in the arrangement of their component cells and are of unusual interest inasmuch as they provide evidence of qualitative and quantitative differences in the constituent blastomeres. They provide also some evidence which may explain the manner in which the central cell becomes enclosed. An undoubted central cell is present in no. 12C.

Unfortunately, however, we have no record of the living ten-cell vitellus. The absence of any photographs of the intact egg, however, may be taken as evidence that it differed but little in appearance from its litter mates. The living twelve- and thirteen-celled eggs are very similar in appearance to those with nine cells (Pl. 1, fig. 12 and Pl. 2, fig. 13).

Ten-cell stage. In the granular cytoplasm of the blastomeres of the sectioned egg (Pl. 4, figs. 37, 38) fat vacuoles of varying sizes are found concentrated around the margins of the cells bordering the zona pellucida where the nuclei are situated. In some of these, however, the lacunae of the fat globules are larger and much more abundant than in others (e.g. that on the left in fig. 37), and in these instances tend to be more evenly distributed throughout the body of the cell. None of the nuclei are in mitosis: all, however, are irregular or bilobed, and a polar body with a large and distinct chromatin mass lies between the central borders of the cells. The occurrence of a ten-cell stage, with nuclei fully constituted and at rest, indicates that cleavage of the eight cells of the eight-cell stage is not always simultaneous, and two of the cells in this instance must be regarded as having divided much sooner than the rest. An inspection of Table 3 reveals an unmistakable disparity in the volumes of the constituent blastomeres of this egg (no. 12B). The largest cell, A, contains 14·7 % of the total volume of the cytoplasmic material of the egg, and is almost three times as large as the smallest J, which contains 5·88 %. This disparity is even more striking than in the eight-cell stage. As yet, however, except for differences in size and a more liberal quota of fat globules in one cell, all the cells appear to be similar.

Twelve-cell stage. Three views of the model of egg no. 12A with twelve blastomeres are shown in Text-figs. 3 *c*, *d* and 4 *a* (section). This egg differs from the one last described in the following respects: (1) in addition to an increase in the number of its constituent cells, one large cell (5 in the models) is nearly completely enclosed, only the outer end of this cell being visible through a gap in the side of the model (Text-figs. 3 *c*, 4 *a*) bounded by blastomeres 1, 2, 3 and 4; (2) the inner half of blastomere 2, also a large cell (Text-fig. 4 *a*), is completely enclosed from above, below, and on each side by the neighbouring cells, while the outer half projects as much beyond the periphery as the inner half projects into the centre of the egg. Unfortunately, however, the nuclei of these two cells (Pl. 4, fig. 39), as indeed of all the cells of this egg, are in the resting condition, and we are unable to state precisely what the axis of the mitotic figure would have been had these cells been allowed to divide. Nevertheless, in view of the condition of the nuclear divisions in some of the larger cells of later stages, we venture to suggest that these cells would divide in such a way that the inner of the two daughter cells would be completely enclosed; one cell being in advance of the other, a thirteen-cell stage would result. Such a condition is established in the next egg to be described. As in the ten-cell stage the cells are similar in appearance (Pl. 4, figs. 39, 40) and apart from size and form no cytological differences could be detected between them. The only differences which are sufficiently marked as to be interpreted as evidence of the early differentiation of the trophoblast are those relating to differences in the size and form of the cells, those in the lower half of the figures being perceptibly smaller and more flattened than those in the upper half of the figures. A study of Table 3 (12A) reveals a discrepancy in the size of the blastomeres which is even more marked than in the last egg. There are two large cells, four small, and six of about medium size. The two largest, A and B, take up 13·85

and 12·04% of the total volume, while of the four smallest cells three (I, J and K) are equal in size and contain 6·02% of the total volume, the smallest, L, having a volume percentage of 3·01. The remaining cells have percentage volumes of 9·63, 9·04 (four cells) and 7·22. From these data it is evident that blastomere A is more than four times as large as L, and B is exactly twice the size of I, J and K and three times as large as L. This discrepancy in the volumes of the individual cells is not without ontogenetic significance, and will be taken up when the thirteen-cell egg has been considered.

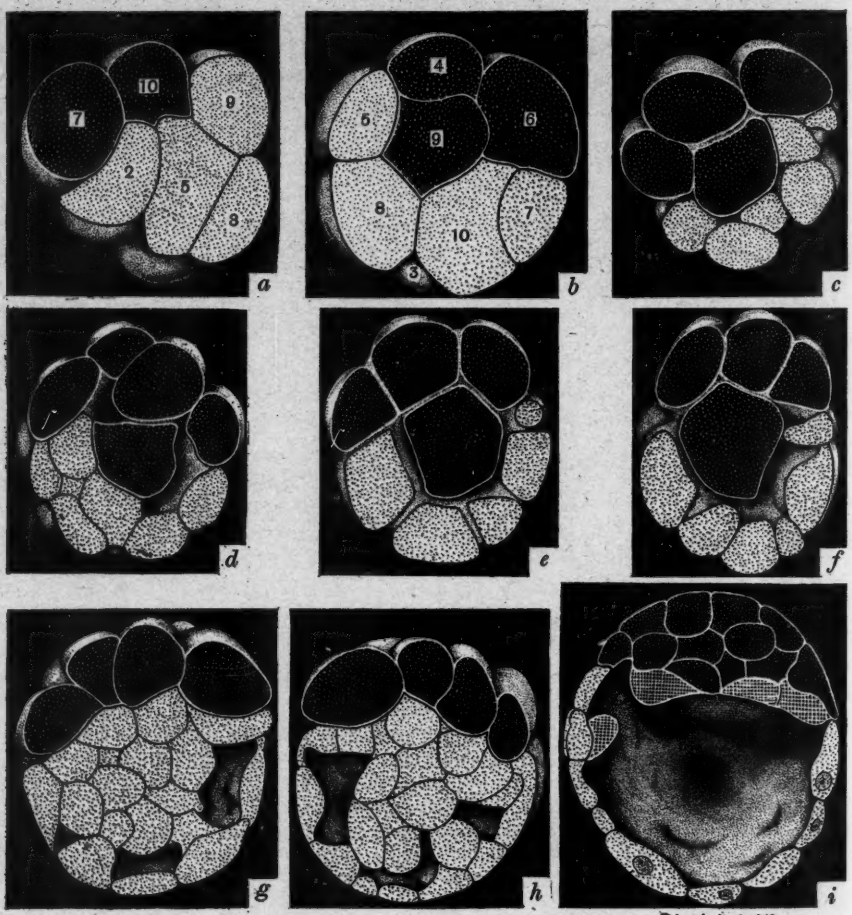
Thirteen-cell stage. This morula no. 12C, of slightly greater diameter than the preceding (Table 1, 12C), has one of its blastomeres completely enclosed (9 in Text-fig. 4 *b*) and is distinctly more advanced in development. Pl. 2, fig. 13, shows its appearance in the fresh condition in saline, and a view of a section of the model of this egg is shown in Text-fig. 4 *b*, while a section through the mid-plane of the ovum is illustrated in Pl. 4, fig. 41. In the intact condition it is very similar to the twelve-cell stage (cf. Pl. 1, fig. 12). The egg now consists of thirteen blastomeres, twelve superficial and one undoubtedly central (Text-fig. 4 *b* and Pl. 4, fig. 41). On examination of the cut surface of the model, it is found that cell 9, having a percentage volume of 4·5, is completely enclosed, and it is in contact with five peripheral cells (4, 5, 6, 8 and 10). Of these, cell 10 with a volume percentage of 9·60 projects deeply into the central mass of the egg and its base is almost completely surrounded as in the case of cell 2 in the twelve-cell egg no. 12B (Text-fig. 4 *a*). From its size and relations it seems very likely that its inner end was destined to become enclosed when the cell divided. There is, however, no indication that division is impending, since all the cells of this egg have resting nuclei and we are, consequently, unable to state precisely what the results of division would be. In the sectioned egg (Pl. 4, fig. 41), all the cells are alike in structure and have an appearance similar to the cells of the twelve-cell stage, except that the larger fat globules are more evenly distributed about the outer margin of the cells. The cells are compactly arranged. The nuclei, however, are central and, in contrast to some of the resting nuclei found at earlier stages, are no longer lobed. As in the twelve-celled egg, although the blastomeres are histologically alike, they show considerable differences in size (Table 3, 12C); two cells (A and B) are larger than the others, two (L and M) are small, while the nine remaining may be arranged in five groups which form an evenly graded series between the two extremes. This disparity in cell size which is so marked a feature in the eggs of this litter is not without significance and, as has been shown by Heuser & Streeter (1929), points to the more active division on the part of some cells than others. It provides the first concrete evidence of the early differentiation of the trophoblast cells.

Late morulae

Fertile goat eggs soon after they have entered the uterus begin to show unmistakable signs of an increase in the number of central cells and the commencing formation of the cavity of the blastocyst. At the same time, some, but not all, of the peripheral cells begin to show evident signs of flattening out. There are three such eggs in our collection. They were all obtained from a single female precisely 120 hr. after copulation and are figured in the living state on Pl. 2, figs. 14–16. Two, nos. 7A and 7B, had just entered the uterine horn, while the third (no. 7C) was still at the tubo-uterine

junction. Only 7A and 7C are available in the sectioned series, 7B having been lost during embedding.

The living eggs exhibit varying degrees of compactness or adhesion of the cells to one another. In some (e.g. fig. 15), where the surface tension is presumably high, the



Text-fig. 4. Models of goat eggs showing central cells, differentiation of formative cells (black with white stipple), trophoblast (stippled), and entoderm (cross-hatched), and origin of the blastocyst cavity. × c. 460. *a* (section), egg no. 12A, twelve-cell stage; *b* (section), egg no. 12C, thirteen-cell stage with central cell; *c*, *d* (cut surfaces), morula no. 7C, thirty-cell stage; *e*, *f* (cut surfaces), morula no. 7A, thirty-two cell stage; *g*, *h* (cut surfaces), early blastocyst no. 13B, about ninety-six cells; *i* (section), blastocyst no. 8, about 126 cells.

blastomeres are spherical and project beyond the general level of the egg, while in others (e.g. fig. 16) there is the opposite extreme, for the cells are closely adherent and form a more compact mass. In Table 1 (7A, 7B and 7C) are given the measurements of the living egg, and it is to be noted that the mean inner diameter of the

cavity of the zona of all these eggs is, exceptionally, slightly smaller than in the remaining eggs of the series, while the zona pellucida itself is, in the case of eggs 7A and 7B, perceptibly thicker.

Of this series 7C is a spherical morula recovered from the tubo-uterine junction and consists of a solid mass of thirty cells of varying sizes, which are separated by a large perivitelline space from the zona. Four of these are centrals, one of which is larger than the others and possesses a large chromatic nucleus (Pl. 4, fig. 44). The others are smaller and have perceptibly smaller nuclei (Pl. 4, figs. 42, 43). The blastomeres are not so compactly arranged as in the preceding stages, the central cells, in particular, being separated by obvious intercellular spaces. These cells have all well-defined outlines and are cuboidal or irregularly polygonal, and what we take to be the commencing blastocyst cavity occurs as a series of irregular cleft-like spaces between the centrally placed cells and peripheral cells. Two views of the cut surfaces of the model of this egg, showing the relationship of these spaces to the peripheral and central cells, are illustrated in Text-fig. 4 *c*, *d*. Apart from size and position, the cells show no marked cytological differences, although those beginning to flatten on the periphery are obvious trophoblast cells.

These peripheral cells are clearly marked off from the centrals and vary greatly in size. The larger cells (shaded dark in the models) occupy one pole and are adherent in places to the centrals, while the smaller bound the incipient cavity and are found at the opposite pole of the cell. Some of these cells have numerous fatty globules which are responsible for the vacuolated appearance of the cells seen in Pl. 4, figs. 42–44, indicating the sectional anatomy of this egg. A number are in varying stages of mitosis. One cleavage spindle in a peripheral cell of average size (that in the upper right quadrant of the figure) which is of interest is shown in Pl. 4, fig. 44. One pole of the spindle, in anaphase, is directed towards the large central cell, the other towards the periphery, so that on the completion of the division two cells would result of which one would be a central and the other a peripheral. We are of opinion that central cells in this and in previous stages result from the radial division of these more peripherally lying cells.

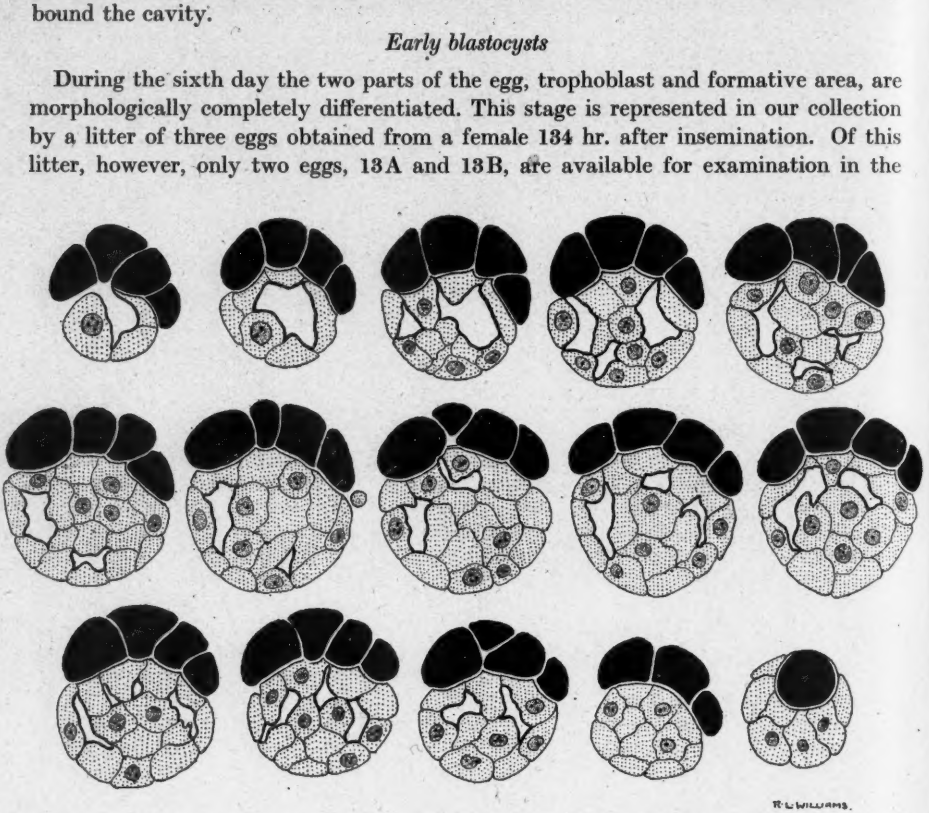
Our next egg, no. 7A, a litter mate of the preceding, differs in appearance in the living state from its sibling. It shows in the sectional series (Pl. 4, figs. 45–48), as also in the photographs of the cut surfaces of the model (Text-fig. 4 *e*, *f*), a definite advance in the degree of organization of its constituent cells. These have acquired more certain identifying characteristics which develop pari passu with the formation of the cavity of the blastocyst and can now be more readily separated into a group of larger cells occupying one pole (the shaded cells in the model), and a group of smaller cells which lie at the opposite pole.

In this egg there are thirty-two cells; four centrals and twenty-eight peripherals. Of the latter, seven are large and form a compact group on the surface of the egg closely adherent to the central cells. The smaller peripheral cells are ovoid or slightly flattened, with large nuclei and sparse fat globules, and constitute a continuous investing layer over the lower hemisphere of the egg. The central cells are irregularly polygonal and vary in size (Pl. 4, figs. 45–48). The largest is in mitosis—in anaphase—with the axis of the spindle parallel to the group of seven large peripheral cells and would, on completing its division, yield two cells of which both would remain in contact with the larger surface cells. The blastocyst cavity is again repre-

sented by a series of broad intercellular spaces between the centrals and the smaller peripheral cells, and extends from the lower pole of the egg to just past the equator (Text-fig. 4 *e, f*). The flattening of the peripheral cells is more evident where they bound the cavity.

Early blastocysts

During the sixth day the two parts of the egg, trophoblast and formative area, are morphologically completely differentiated. This stage is represented in our collection by a litter of three eggs obtained from a female 134 hr. after insemination. Of this litter, however, only two eggs, 13A and 13B, are available for examination in the



Text-fig. 5. Tracings of photomicrographs (× 320), of serial sections of egg no. 13B, at 134 hr. post-coitum, showing the demarcation of the trophoblast (stippled) and the residual formative cells (black). The blastocyst cavity is represented by a series of fluid-filled intercellular spaces in the trophoblastic part of the egg. The cellular mass of the trophoblast which is adherent to the under-surface of the formative cells contributes to the formation of the entoderm.

serial sections, the other having been lost in handling. These two eggs are essentially similar in appearance, differing only in the number of blastomeres and the extent of development of the blastocyst cavity. Pl. 2, figs. 17, 18, portray eggs no. 13C (the one which was lost) and no. 13B in the living state. As can be seen from the photographs of the living egg, marked structural changes have taken place since the last stage, and a crescentic group of larger cells is present at one pole (Text-fig. 4 *g, h*) while a sharply defined group encircling a central darker area (the cavity of the blastocyst) occupies the other pole. The egg thus appears definitely regulated as to its axis, and we can identify with certainty the two groups of cells which are destined to form the formative area and the trophoblast and, coincident with the differentiation

of the latter, the blastocyst cavity. It is noteworthy that in both the eggs of this stage where the cavity of the blastocyst is better defined, the blastocyst wall is closely applied to the inner surface of the zona and is not, as in the previous stage, separated from it by a considerable space.

Since it was possible, because of the clear-cut separation of the groups of cells, to orientate the egg as to the plane of cutting, the sectional series (Pl. 5, figs. 49–52) amply confirm the demarcation between the trophoblast cells and the larger formative cells at the embryonic pole. The series also provides evidence of the increase in size of the intercellular spaces between the trophoblastic elements. As these features are more evident when the sections are examined in series, serial tracings of egg no. 13B, in which the trophoblast is shown in stipple and the formative cells in black, are reproduced as Text-fig. 5. Examination of this series suffices to carry conviction of the completeness in the separation of the two groups of cells and the acquisition by the egg of its distinctive polarity.

Turning now to the actual developmental transformations of the blastocyst cavity, it is found to be perceptibly larger than in the antecedent stages and now has the form of a curved intercellular cleft between the trophoblast cells, so situated as to separate a single layer of trophoblast, which clothes the under-surface of the formative mass, from the main trophoblast at the lower polar region. As regards the manner of formation, there seems little doubt that its enlargement has been brought about by the confluence of the fluid-filled intercellular spaces of the earlier stages.

A count was made of the cells in both of the sectioned eggs of this stage, and their approximate constitution was as follows:

Egg 13A consists of eighty cells of which twelve constitute the formative area and sixty-eight are trophoblastic, while egg 13B consists of ninety-six cells, of which nineteen are formative (some distinctly larger than others) and seventy-seven trophoblastic; and of the latter sixteen form the roof of the cavity of the blastocyst and are closely applied to the under-surface of the formative mass.

The didermic blastocyst

There is, unfortunately, a gap between the stage just described and our uterine blastocyst no. 8, which is the last of the series. This egg was recovered 156 hr. after copulation. Pl. 2, figs. 19–21, portray the egg in its entirety, while Text-fig. 4 *i* shows a cut surface of the model of the egg.

The blastocyst is now fully constituted and its polarity unmistakable. It consists of an outer investing layer of trophoblast composed of a single layer of loose flattened cells and an inner cell mass at the embryonic pole of many compact cells, the majority of which are rather larger and more irregular than those constituting the outer wall. The spherical zona is still intact and there are no indications of impending disintegration. It has an inner diameter of 145 μ, an outer of 170 μ and a thickness of 12·5 μ, measurements which are identical with those of our fertile unsegmented egg. The formative mass (Pl. 5, figs. 53–60) measures on the slides 0·098 × 0·033 × 0·075 mm. and has the form of a compact ovoidal disc composed of rather large, deeply staining, irregular cells; while overlying these are a few small irregularly shaped cells which blend with the flattened parietal trophoblast elements at the margins of the disc. They are the covering trophoblast. These covering cells cannot always be identified overlying the larger formative cells (see Pl. 5, figs. 53, 54), and it seems highly probable

that the former are increased at the expense of cells and their descendants now forming a portion of the formative mass; in other words, they are in process of being differentiated off from the primitive large cells. In contact with the lower surface of the disc there are present, especially at one side, cells of a more elongated form and rather different appearance, which may be regarded with some confidence as the representatives of the future entoderm. It is noteworthy that this precocious segregation of cells from the under-surface of the disc is coincident with the complete differentiation of the cavity of the blastocyst and so with the trophoblast.

The primitive entoderm cells are very similar in appearance to the cells of the parietal trophoblast, and appearances such as those shown in Pl. 5, figs. 56, 57, where a connecting strand of cytoplasm may be seen passing from a parietal cell to one on the under-surface of the disc, suggests a continuity of origin between the two. At the other end of the disc, Pl. 5, figs. 53, 54, it is impossible to be certain whether a cell is being incorporated into the disc or whether it is migrating therefrom. Here is evidence, then, of a contemporary origin of primitive entoderm and trophoblast and, by inference, an independent segregative origin of entoderm and formative cells. We venture to suggest, therefore, that these primitive entoderm cells are the direct descendants of some such cells of the trophoblast as those which roofed the incipient cavity in our last egg. Finally, when a count was made of the numbers of cells in this egg, its approximate constitution was as follows: (1) formative cells, 40; (2) primitive entoderm, 15; (3) covering trophoblast, 22; and (4) parietal trophoblast, 59.

COMMENTS AND COMPARISONS

Egg size

For very few ungulates do we have adequate and comparable measurements of the living egg. The few available measurements are summarized in Table 4, while Tables 1 and 2 give the results of our own measurements on the sixteen goat eggs examined in the present investigation.

Table 4. *Comparative measurements of living ova (ungulate)*

Animal	Investigator	Outside diameter of zona in μ	Inside diameter of zona in μ	Thickness of zona in μ
Cow	Evans & Miller (1935)	192	150·7	20·6
	Hartman *et al.* (1931)	167	140	13·5
	*R. V. C. (unpublished)	162	137	12·5
Sheep	Assheton (1898*a*)	180	150	15·0
	Allen *et al.* (1931)	167	143	12·0
	Clark (1934)	175	147	14·0
	R. V. C. (unpublished)	180	153	13·5
Goat	Amoroso *et al.* (present investigation)	170·4	145·3	12·5
	Amoroso *et al.* (1939*b*)	170	145	12·5
	Hartman (1929)	—	140	—
Pig	Assheton (1898*b*)	164	132	16·0
	Heuser & Streeter (1929)	160	130	15·0
	R. V. C. (unpublished)	157	132	12·5
Horse	Amoroso *et al.* (1939*a*)	158	131	13·5
	Hartman (1929)	—	135	—

* The unpublished data is derived from measurements of the series of ova in the possession of one of us (E. C. A.).

The indications from these data (see Table 1) are that goat eggs, except for the transient but perceptible diminution in the diameter of the zona cavity in the late

morulae (7A, 7B and 7C), do not vary considerably in size during the period when the 'formative' and 'trophoblastic' parts of the egg are in process of differentiation, and the average of 145·3 μ for our series of eggs is remarkably close to the average figure of 147·0 μ obtained by Clark (1934) for his series of sheep ova. Furthermore, it is evident that while the absolute size (see Table 4) of the mature ovum varies from species to species there is, in general (especially among the Pecora), a remarkable degree of similarity in the different eggs as measured by the inner diameter of the zona pellucida. In all of the investigations made upon the ungulates, only in the case of the pig and horse, animals with an epithelio-chorial placenta, is there any divergence in egg size from the tabulated mean for the Pecora. The associations demonstrated by the tabulated data cannot be accidental, and they cannot depend upon the size of the animal. It seems probable, however, that they may depend in some way upon the mechanism for implantation, and the relationship of egg size to placentation may be a primary one.

Zona pellucida and cumulus

From the photographs of the living egg and from Table 1 it is evident that the zona pellucida in the goat remains fairly uniform in thickness during its tubal sojourn. Even as late as our blastocyst stage it retains many of the characteristics of the earlier stages, and it has almost the same dimensions and the same homogeneous appearance. How long it remains intact in the goat we are unable to state.

No corona radiata cells were attached to the zona in any of the goat eggs examined. This absence of adherent cumulus cells has also been recorded for the roe (Bischoff, 1854), sheep (Assheton, 1898*a*; McKenzie & Allen, 1933; Clark, 1934; McKenzie & Terrill, 1937; and others), cow (Hartman, Lewis, Miller & Swett, 1931; Evans & Miller, 1935), pig (Assheton, 1898*b*; Corner & Amsbaugh, 1917; Corner, 1917; Heuser & Streeter, 1929), and horse (Amoroso *et al.* 1939*a*). Whether corona cells are still in position at the time of ovulation, disappearing soon thereafter, or whether the ovum lies unattached to the surrounding cumulus just before the follicle ruptures, is at present uncertain. On the one hand, Webster (1921) refers to the flattened cumulus, about the size of a pin head, on the relatively enormous wall of the cow follicle, while Allen *et al.* (1930), on the other hand, have recorded a well-organized corona about an ovum from a large follicle from a human ovary. The persistence of a well-developed corona radiata surrounding the freshly ovulated egg occurs with regularity not only in the rabbit (Gregory, 1930; Pincus & Enzmann, 1932), guinea-pig (Squier, 1932), rat (Gilchrist & Pincus, 1932) and mouse (Lewis & Wright, 1935), but also in the cat (Hill & Tribe, 1924), dog (Evans & Cole, 1931), ferret (Hamilton, 1934) and man (Allen *et al.* 1930). It is evident, therefore, that, while in many mammals the ova are shed surrounded by an apparently sticky cumulus, the ungulates appear to be exceptional, since very few or no follicle cells surround the egg soon after it is shed. The reasons for this species difference are not known, though the exceptional rate of tubal transit of opossum ova which enter the uterus in approximately 24 hr. has been attributed to their lack of surrounding cumulus (Pincus, 1936). While the absence of any adherent cumulus may be invoked as the cause of the acceleration of tubal transit in the opossum, and possibly also the pig, it is clear that we cannot associate this phenomenon causally with the duration of the tubal journey in the goat and sheep; for the eggs of the

latter, which are usually devoid of adherent cumulus and have a shorter distance to travel, require a much longer time for the tubal passage than do the eggs of the pig.

In its morphology the goat egg is in all essentials similar to those of the other ungulates so far described. The cytoplasm of the egg is remarkably clear and contains fine fat granules evenly distributed throughout the egg. In this respect it resembles more closely the sheep egg than those of the pig and horse which are more opaque and possess coarser fat droplets. There are no indications of polarity in the uncleaved egg. The unicellular vitellus is spheroidal or nearly so, and does not fill the space of the zona. Within this perivitelline space a polar body is invariably present. In a few cases two were seen in the sectioned egg. The polar bodies are usually small, compact and spheroidal, though ovoid forms were encountered. They vary greatly in position in the different eggs, sometimes being found in the superficial grooves between the blastomeres, at other times buried deeply in the spaces enclosed by the central margins of the cells. There can be no doubt that this variation in position of the polar bodies is due to segmentation, pressure, and readjustment (Gregory, 1930). The almost universal occurrence of a single polar body (presumably the second, since the great majority of the eggs are fertile) in the segmenting goat egg points to the early disintegration of the first body. This is in agreement with the conclusions of Lewis & Wright (1935), who found that in more than 50 % of their mouse eggs the first polar body had disappeared by the four-cell stage, while in only 25 % of the eggs were two bodies present in the eight-cell and morula stages. It is of some interest in this connexion to find that although Allen, McKenzie, Kennedy & Beare (1931) identified polar bodies in several sheep ova, neither Assheton (1898*a*) nor Clark (1934) were able to find them; nor do Green & Winters (1935) and McKenzie & Terrill (1937) figure them. In the two-cell egg of the cow described by Hartman *et al.* (1931) 'neither polar body was differentiable'. In the goat a polar body is still identifiable in our late morulae, as indeed in the blastocyst we describe.

Age, stage and location of eggs

Table 5 summarizes our observations on the age, state of development, and location of the several cleavage stages and early blastodermic vesicles. The ages given refer to the post-coital age, and since we have no definite information of the time relation between copulation and ovulation, it will be readily understood that the ages are only approximate. Up to 30 hr. post-coitum the egg remains unsegmented, but is preparing to divide as is evidenced by the formation of the first cleavage spindle. It will be recalled that egg 4A (recovered 30½ hr. post-coitum) completed the division from the one-cell to the two-cell stage in the interval which elapsed between recovery and photography. In our other two-cell specimen recovered from the tube 48 hr. after copulation, division of the blastomeres is imminent. That the two-cell stage lasts about 18 hr. would seem, therefore, a permissible deduction. Eggs from 50–60 hr. post-coitum are in the four-cell stage, while those recovered between 60 and 85 hr. have completed the third cleavage while still in the fallopian tube. Older eggs, morulae, and early blastocysts from 98 hr. post-coitum, are at the tubo-uterine junction or have entered the uterus. Goat eggs, therefore, seem to enter the uterus some time between the close of the fourth day or the beginning of the fifth day after mating.

If we seek an orientation in comparative studies on the rate of cleavage of tubal eggs among the ungulates, we find many suggestive but few unequivocal data. This

has been due in part to the fact that, of the forms studied, all ovulate spontaneously and there is, in consequence, some difficulty in timing ovulation. Nevertheless, for four species, pig (Heuser & Streeter, 1929), sheep (Clark, 1934), cow (Hartman *et al.* 1931) and goat (own data), we can construct approximate growth curves. These are presented in Text-fig. 6. It is at once evident that there are striking differences in the rate of cleavage in the four species considered. Thus, while the goat and cow curves are the most regular, being almost straight lines, the sheep is the most precocious in its cleavage rate, and the pig falls somewhere between the two extremes. Further, whereas the first two cleavages occur earlier in the goat than in the pig, thereafter the rates of cleavage are reversed, the pig being the earlier to attain the sixteen-cell and subsequent stages. That this difference in the rate of segmentation is inherent in the eggs is supported by the studies of Castle & Gregory (1929) and Gregory & Castle (1931), on genetic races of rabbits which differ considerably in size,

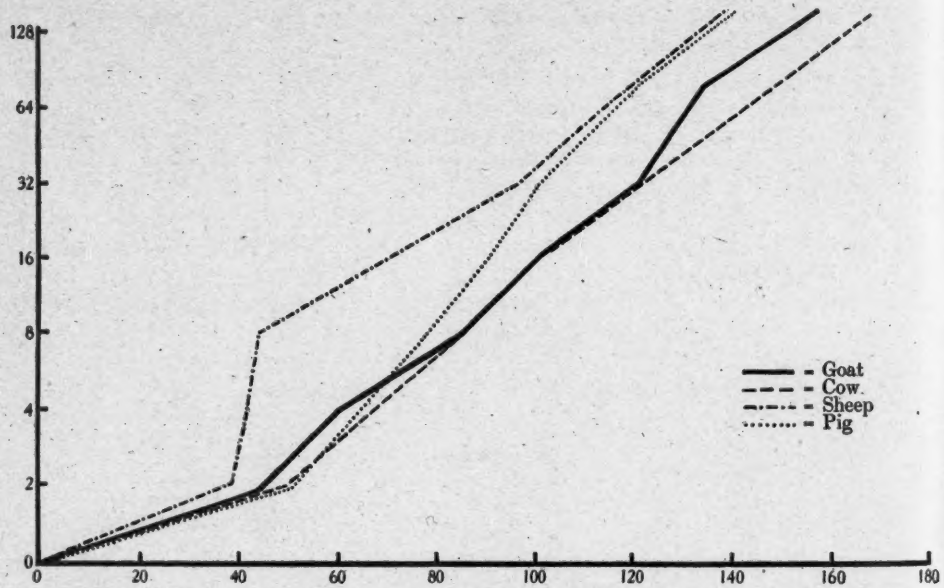
Table 5. *Age, stage and location of eggs*

Female	Egg	Time since mating hr.	Cell stage	Location of eggs in genital tract	Remarks
4	4 B	30½	1	Tube	First cleavage spindle
4	4 A	30½	2	Tube	
1	1 A	48	2	Tube	Second cleavage spindle
5	5 A	60	4	Tube	
	5 B	60	4	Tube	One cell in division
2	2 A	85	8	Tube	All cells with resting nuclei
	2 B	85	8	Tube	
12	12 B	98	10	Uterus	
	12 A	98	12	Uterus	Formation of central cells
	12 C	98	13	Uterus	Central cells
7	7 A	120	32	Uterus	Morulae with central cells and commencing formation of cavity of blastocyst
	7 B	120	30±2	Uterus	
	7 C	120	30	Tubo-uterine junction	
13	13 A	134	80	Uterus	Egg definitely regulated as to axis. Formative area distinct
	13 B	134	96	Uterus	
14	14 A	140	1	Uterus	Degenerate
	14 B	140	9	Uterus	Retarded
8	8 A	156	126	Uterus	Blastocyst

and in which they have shown that the cleavage rate is consistently more rapid in the large-sized race. That they are independent of tubal environment is rendered probable by the corroborative data from rabbit eggs cultivated in vitro (Lewis & Gregory, 1929), where divisions are well within the normal periods.

In addition to the varying rates of cleavage already noted, it must further be pointed out that ungulate eggs differ widely in the times of onset of visible differentiation. Thus, Heuser & Streeter (1929) found that differentiation of the trophoblast in the pig begins as early as 4 days 3½ hr. after copulation, at which time the egg has left the tube and is in about the sixteen-cell stage. In the sheep, on the other hand, Clark (1934) detected a more precocious differentiation of the trophoblast in tubal eggs with sixteen cells at about 65–77 hr. after coitus. The eggs of the goat appear to begin their differentiation late in comparison with those of the sheep and pig; the really decisive evidence for this conclusion, however, is not yet at hand, and the intermediate stages between thirteen and thirty cells will be required for a definite

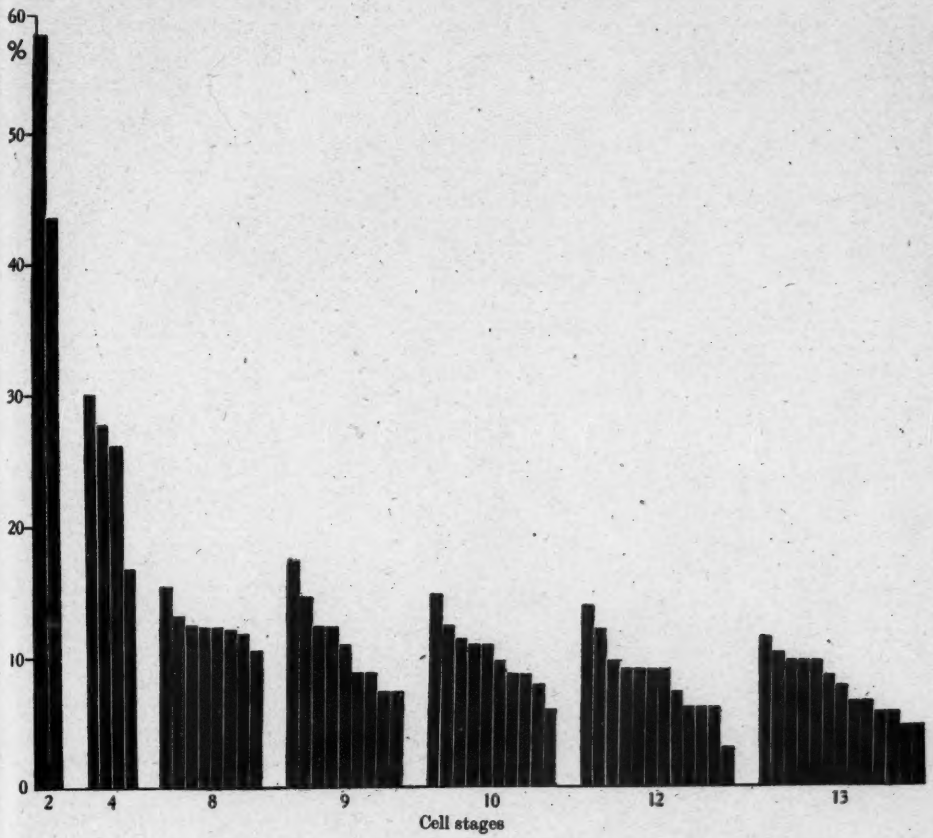
solution of the problem. In the common stock rabbit Gregory (1930) reports that separation of the trophoblast from the rest of the egg is early, unquestionable signs of the change being found in the seventeen-celled stage 41–48 hr. after coitus. Castle & Gregory (1929) and also Gregory & Castle (1931) were further able to show that in spite of the inherent differences in the speed of segmentation in the large- and small-sized breeds the process of differentiation occurs at the same time. This dissociation of the fundamental embryogenic processes, however, has long been known, but to these authors must be given the credit of tracing it back as far as the morula stage. It may well be, then, that the more rapid rate of cleavage of the sheep egg as compared with those of the goat, cow, and pig depends on some such inherent mitotic intensity quite independent of differentiation rates.



Text-fig. 6. Comparative development of ova of the goat, sheep, cow and pig. Abscissa: time in hours after mating. Ordinate: number of cells. The goat, from data of present investigation. The sheep, from data of Clark (1934). The cow, from data of R.V.C. collection. The pig, from data of Heuser & Streeter (1929).

The wide discrepancies found among mammals in respect of the period of arrival of ova in the uterus have long been known, and their significance commented upon. Allen Thompson (1859) was among the earlier writers to comment upon the lack of correspondence between the time of the tubal journey and the length of gestation. That the rate of passage of ova through the tube is independent of the size of the animal and size of the egg seems well established. As we have already noted, it is independent of the length of the tube, except perhaps in the rabbit (Gregory, 1930), where it may be in some way thus correlated. Our own observations on goat eggs lead us to suspect, however, that in the ungulates at any rate, the time of arrival of the eggs in the uterus may, in some measure, depend upon the time at which the

initial segregation of the formative and trophoblastic parts of the egg occurs. The collected data (Table 3 and Text-fig. 7) of the proportional volume constitution of our goat eggs, and more especially of 12 A, B and C (which had just entered the uterus), suggest inequalities in the size of the component cells and the possibility of the occurrence of more active division on the part of some cells than of others.



Text-fig. 7. Relative volumes of blastomeres of two-, four-, eight-, nine-, ten-, twelve- and thirteen-cell stages.

This view appears to us to be supported by the data derived from the study of the cleavage process in the pig (Assheton, 1898*b*; Heuser & Streeter, 1929) and sheep (Assheton, 1898*a*; Clark, 1934). Thus, Heuser & Streeter report that in the case of the pig, at about the time the egg passes from the tube into the uterus, which it does between the close of the second day and the beginning of the third day, it is found in the four-cell stage. At this time the egg shows a distinct disparity in respect of cell size, and, correlated with this, a definite difference in the rate of subdivision of the constituent cells. 'The property', they state, 'of more active cleavage is the first evidence that this egg shows of the sorting and localization of special substances.'

Assheton had already averred as early as 1898 that variability in respect of cell size in pig eggs was pronounced in the four-cell tubal egg, but more so in the later five-segment stage when the egg had just entered the uterine horn. In the sheep he has also described an eight-cell tubal egg in which one segment differs most markedly in texture and in colour from all the remaining segments, while in another egg, also with eight cells, but recovered from 'the upper end of one horn of the uterus of a sheep killed on the fourth day, one cell was slightly larger than any of the others'. Clark has also remarked upon this disparity in cell size of sheep ova just before they enter the uterus. He finds, however, that the discrepancy is sufficiently well marked only when the egg has reached the sixteen-cell stage.

REMARKS ON CLEAVAGE

It is of some significance that certain striking similarities exist in the cleavage pattern of the eggs of the pig, sheep and goat. In the goat the two blastomeres from the first cleavage are unequal in size, but no qualitative differences between the two cells, as is described for the rat (Huber, 1915), dog (O. van der Stricht, 1923) and cat (R. van der Stricht, 1911; and Hill & Tribe, 1924) could be detected. Such a discrepancy in cell size has been reported for a number of mammals and would seem also to be the usual condition among the ungulates, having been recorded for the pig (Heuser & Streeter, 1929), cow (Hartman *et al.* 1931) and sheep (Clark, 1934). That this difference is merely a matter of chance separation of cytoplasmic material in the progress of the initial cleavage is suggested by the work of one of us (W. J. H. 1934) for the ferret; the really decisive evidence for this conclusion is, however, not yet available. In the pig, on the other hand, Heuser & Streeter (1929), though they could find no demonstrable cytological differences between the two cells, tentatively suggested that they were probably not identical, and that with the completion of the first cleavage there had doubtless occurred some sorting of the constituent substances of the egg.

At the second cleavage the divisions are effected in two planes at right angles to each other, and the two blastomeres divide at very nearly, but not quite, the same time, giving rise to a four-cell stage with the blastomeres so arranged as to form an interlocking cross-shaped figure. This cross arrangement of the blastomeres has been described in the pig (Assheton, 1898*a*; Heuser & Streeter, 1929), sheep (Clark, 1934), rabbit (Assheton, 1909; Gregory, 1930), hedgehog (Assheton, 1909), guinea-pig (Squier, 1932), mouse (Sabotta, 1924; Lewis & Wright, 1935), cat (Hill & Tribe, 1924), ferret (Assheton, 1909; Hamilton, 1934), dog (Bischoff, 1845; Assheton, 1909), monkey (Lewis & Hartman, 1931). Macdonald & Long (1934) figure it for the rat and one of us (E. C. A.) has observed it in the cat, dog, pig, sheep and cow. In both of our living specimens the cross-arrangement was definite, but we did not observe any movement of the cells as described for the ferret (Hamilton, 1934). As pointed out by Hamilton the cross-arrangement of the spindles at the two-cell stage might lead us to expect a difference in the appearance of the cells at the four-cell stage. There is some evidence in the goat that this is so, for both our four-celled eggs are noteworthy in that each shows some inequality in the distribution of fat, one pair of cells being richer in fat globules than the other.

As has already been described, the nuclear conditions in the larger blastomere of the two-cell stage is only slightly in advance of that of the smaller cell. Consequently,

in the goat the three-cell stage is abbreviated, the egg passing from the two- to the four-cell stage without the intervention of a pronounced three-cell stage. This fleeting condition of the three-cell stage has also been recorded for the ferret (Hamilton, 1934) and seems also to be true for the cat, for, though R. van der Stricht (1911) described two three-celled eggs, Hill & Tribe (1924) failed to recover this stage in their unique collection of tubal eggs.

Concerning the slight priority of the larger blastomere in the two successive divisions of the second cleavage of the goat egg, it is of some interest to find corroborative data in the motion-picture records of the living monkey egg (Lewis & Hartman, 1931). In the pig Heuser & Streeter (1929) also find that one of the blastomeres is more precocious in the onset of the second cleavage, giving rise to a three-cell egg. However, they do not state in which cell this priority is manifest, but point out that from this stage on the cells can be divided into those which show 'more active cleavage' and those which are 'more deliberate'. These authors attach considerable importance to these differences in size and rate of division, as affording the first evidence of 'the sorting and localization of special substances in the egg', the more actively dividing cells being the precursors of the trophoblast, the more lethargic cells those which give rise to the embryo proper. So far the only visible evidence of differences in the cell groups of our four-cell eggs is a slight variation in the proportionate volume of the cells (see Table 3 and Text-fig. 7), and as far as our observations go we are inclined to think that the early dissociation of the cells, such as exists so constantly and characteristically in the pig, is effected at a later stage in the goat.

In our material there are no intermediate stages between those with four cells recovered 60 hr. post-coitum, and those with eight cells at 85 hr. after mating. Our third cleavage is represented by two eggs at the eight-cell stage, in both of which it is possible to recognize one cell which is constantly large, and one small.

The fourth cleavage is represented in our material by four eggs in the nine-, ten-, twelve- and thirteen-cell stage. An undoubted central cell is present in the twelve-cell egg, and there are indications in the thirteen-cell specimen that the central cells are produced by the radial division of some of the peripherals. In all our eggs of this cleavage we can recognize a polarity in respect of the position of the constituent cells which are segregated into groups of smaller and larger cells occupying opposite poles, and which we interpret as indicative of the more active division on the part of some cells than of others. It will be recalled that this dissociation of the cells was observed in the pig and sheep, and it has also been recorded for the cat, but the time at which it occurs varies somewhat in the different species. Thus, in the pig it is effected early, at the second cleavage; in the sheep at the third or fourth; while in the cat, according to Hill & Tribe (1924), it may be as early as the second and not later than the third. That there is a relation, indeed a correlation, between the time when the egg acquires its distinctive polarity and the time of its arrival in the uterus has already been stressed. Hill & Tribe (1924) and Heuser & Streeter (1929) lay emphasis on this early dissociation of the blastomeres into two groups with determinate destinies. Moreover, Heuser & Streeter believe that the varying rates of division between the groups of cells is the really decisive factor. Furthermore, we believe with Streeter that the forces that appear to be responsible for their segregation are intrinsic and purely genetic rather than environmental. We cannot, however, agree with Hill & Tribe (1924) when they suggest that the process of epiboly, which they believe they have

conclusively demonstrated for the cat, may be of universal occurrence in the Monodelphia, 'though evidently not always easy to demonstrate'. We question the speculation of Hill & Tribe, for which slight evidence is presented. We incline rather to the view (not new) that the central cells arise by radial division of some of the peripheral cells, further increase being aided by division of pre-existing cells. Indeed, if speculation on such meagre grounds is justified, the evident difficulty in demonstrating overgrowth might better be regarded as indicative of its absence.

With the completion of the fourth cleavage the egg is provided with the necessary requirements for the organization of the blastocyst, and by the time the morula consists of thirty-two cells a considerable degree of differentiation has been attained. The cells have now acquired more certain identifying characteristics, and marked functional changes are demonstrably associated with the differences which mark the early recognition of the trophoblast.

The two groups of cells which were recognizable earlier, though with less certainty, are now unmistakable. We have, on the one hand, the large, isolated, slowly dividing, primitive cells at the upper pole of the embryo; on the other hand, there are the smaller, more actively dividing cells which are well advanced in differentiation, and form an attenuated investing layer over the lower hemisphere of the egg. In contrast to these are the centrals. Some are large undifferentiated cells which repeat the structural design of the primitive cells, and with these they constitute a group. Others are smaller, further advanced in differentiation, and in places are separated by wide intercellular spaces from the flattened cells with which they thus present relations of varying intimacy. This distinction between the two types of centrals is stressed, because while at this stage they represent the closest associations of individuals, their further history shows that they are not equivalent groups. Moreover, the subsequent stages of development of the goat justify us in recognizing in the smaller cells trophoblastic elements, from which the parietal trophoblast and part of the entoderm will arise, and in the larger cells the formative elements which, after giving origin to covering trophoblast, are destined to lose their individuality and co-operate in the formation of the embryonic shield. Our goat morulae thus reproduce in all essentials the conditions described and figured by Heuser & Streeter (1929) for their pig eggs with sixteen to twenty cells (cf. their fig. 6 *g*, with our Text-fig. 4 *g*, *h*).

In our early blastocysts 13A, 13B and 13C with eighty to ninety-six cells, the continuous but independent mitotic rhythms of the trophoblast and formative cells have produced the characteristic segregation of the two parts of the egg. These eggs also provide evidence of the way the entire groups of cells exercise directive influences upon the further course of development of the blastocyst. In the eggs of this litter the blastocyst cavity appears in the form of a crescentic intercellular cleft in the trophoblastic part of the egg and is so situated as to separate a single layer of trophoblast which remains adherent to the mass of laggard cells from the main trophoblast—which may in places be several layers thick—at the lower polar region of the embryo. As a result, a large part of the main mass of trophoblast cells remains peripheral and gives rise to the parietal layer; the remainder, which are in contact with the formative cells, giving origin, we believe, to primitive entoderm and probably also to parietal cells. In the formation of the blastocyst cavity there is no evidence of liquefaction of the cells, the cavity being entirely intercellular and bounded on all sides by trophoblast. These findings are in general agreement with those of Assheton (1898 *a*, *b*) for

the sheep and pig, and provide confirmation also of the views he advanced for the goat (1908, 1909). While he is inclined to think that the blastocyst cavity, which he regards as the archenteron in contradistinction to the segmentation cavity, owes its origin to the extension of intercellular vacuoles in the pig, it is 'less clearly so in the sheep, and from general appearance still less in the goat'; he is certain, however, that 'in each case it begins as a split amongst the hypoblast (trophoblast) cells'. He states further 'that there are in the sheep, pig, ferret, goat (Assheton, 1908, fig. 5) strands of protoplasm which connect the inner lining of the inner mass to the wall of the blastocyst, and this tends to show that the inner lining of the inner mass is of common origin with the wall of the blastocyst; that is to say, the hypoblast and trophoblast are one'. Recently, Clark (1934) has examined the condition in the sheep, and has interpreted his results as supporting the view of Assheton regarding the hypoblastic origin of the trophoblast.

In their pig egg no. 248–8 with about twenty cells, Heuser & Streeter (1929) have figured and described the demarcation between the characteristic trophoblast cells enclosing the segmentation cavity and the residual primitive cells. Comparison of their figs. 5 and 6 *h* and figs. 26 and 27, pl. 1 with our Text-figs. 4 *g*, *h*, 5, and Pl. 5, figs. 49–52, suffices to carry conviction of the essentially similar character of the underlying structural condition. In their fig. 6 *h* of the cut surfaces of the model of this egg and their figs. 26 and 27, which are apparently consecutive serial sections through the same egg, they certainly show a layer of trophoblast stretching across the under-surface of the primitive mass; but this is not illustrated in the model (fig. 6 *i*) of their next succeeding stage, nos. 291–5; nor is it described in connexion with fig. 31, pl. 2, which is a section through the greatest thickness of the primitive mass of this embryo. What has become of this layer is not stated; but if we may judge from appearances such as are shown in their fig. 26, pl. 1 (no. 284–8), where a cell from the under-surface of the primitive mass appears to be in process of incorporation into the outer wall of the blastocyst cavity; and in fig. 32, pl. 2 from their egg no. 291–6, where 'the first free entoderm mother cell' with its thinned-out process is nearly connected to the inner cell mass, it seems on the whole at least highly probable that:

(1) In the younger embryo no. 284–8, cells of the wall of the blastocyst are being increased at the expense of trophoblast cells now forming a part of the under-surface of the primitive mass.

(2) The cellular tissue, to which the first mother entoderm cell appears to be attached, could be regarded as simply a persisting portion of the cellular mass of the trophoblast which had lost its connexion with the outer layer through the development of the blastocyst cavity. That this layer would perhaps be a network of cells rather than a continuous sheet at the time when the blastocyst cavity is very rapidly expanding, as first suggested by Assheton, seems a permissible deduction.

(3) The accretion of material by the wall trophoblast would also be necessitated by the rapidly increasing size of the vesicle.

(4) On this basis the older blastocyst no. 291–6 would be immediately derivable from the prior condition which is found to exist in no. 284–8.

Heuser & Streeter do not interpret the changes observed by them in terms of the separate layer of trophoblast contributing to the primitive entoderm, but their facts readily fit themselves into that interpretation. They are inclined, however, to think

that the trophoblast does contribute some entoderm, for they state: 'When more mature entoderm cells make their appearance with long processes, they are found adhering to all parts of the blastocyst wall. Often we could not say but that they were arising directly from the trophoblast. However, in view of what has gone before one must not run the danger of being misled by such appearances without more satisfactory proof.'

Returning now to our blastocyst we find that it has acquired its chief identifying characteristic, that is, the trophoblast constituting the larger part of the blastocyst is present as a continuous layer and forms a thin-walled fluid-filled vesicle. At the same time, the inner cell mass has the form of a concavo-convex disc consisting of large irregular formative cells closely invested over the greater part of its outer surface by the covering trophoblast, and on its inner surface by a tenuous membrane of flat primitive entoderm cells. It is evident that in this embryo we are again dealing with a mass of cells essentially similar in character to those already described in connexion with our earlier blastocyst. Furthermore, we are convinced that the covering trophoblast cells are derived from the larger formative cells, evidence of which is provided by the occurrence in our embryo no. 13B, of a few small cells superficially placed among the larger cells (see Pl. 5, figs. 51, 52). This finding is corroborated by the exact observations of Heuser & Streeter (1929) who reported the addition of many cells from the inner cell mass to the trophoblast. We have found no evidence in any of our goat eggs of the 'over-growth' which Assheton (1898*a*) states is 'quite unmistakable in the sheep', of which there is a 'distinct suggestion in the pig', but of the occurrence of which he (1908), oddly enough, assumes in the goat. It is noteworthy, however, that when dealing with the earliest of the goat blastocysts which he describes, Assheton found no certain means of identifying the ectoderm cells (formative) which he believed to be 'imbedded among or between the future trophoblast and hypoblast cells'. But if we may judge from his fig. 1 (Assheton, 1908), it seems clear that while the hypoblast is in the form of a continuous sheet, the covering trophoblast has not yet appeared over the whole extent of the surface of the inner mass. Indeed, Assheton himself provides the evidence. He states (p. 211) 'the cells resemble those of the sheep's blastocyst rather than the pig's in being larger, more clearly delimited and more homogeneous', a description which we believe applies more aptly to large undifferentiated formative cells rather than to trophoblast.

Concerning the inner lining cells, the facts previously alluded to indicate that this network of cells which attaches to the under-surface of the inner cell mass and resembles so closely the cells of the parietal trophoblast, is, at least in part, the remains of the original trophoblast lining, and we are led to believe that it represents the precocious beginnings of the entoderm. The above should not be interpreted, however, as a rejection of the hypothesis that these primitive entoderm cells may not in part be derived from the wall trophoblast, and there are appearances which support the latter view. But even if we assume that some of these cells are not all descendants of the original trophoblast layer which clothed the under-surface of the formative mass, but have a common origin from the parietal trophoblast, the validity of our case is in no way altered. We are compelled, therefore, to admit that the inner cell mass as here constituted is morphologically equivalent to much more than that portion formed at an earlier period and contains derivatives of both formative cells and trophoblast, i.e. covering trophoblast and entoderm, but as yet contains no true ectoderm, for it

is only when the last trophoblast cells are given off that we can speak of the residual formative cells as ectoderm. Thus considered, this prior differentiation of the entoderm. in contradistinction to the ectoderm must leave us ill content to ascribe to the latter the sharp individuality so long conceded to it.

SUMMARY

1. An account is given of the segmentation of the egg of the goat.

2. The vitellus of the living unsegmented egg is composed of fatty globules embedded in a dense mottled matrix and almost completely fills the space within the zona pellucida.

3. No corona cells were attached to the egg in any of the stages examined.

4. A polar body was present in the majority of the ova. Sometimes two were seen.

5. Data are given of the diameters of the different eggs, relative volumes of the blastomeres, and the age stage and location of the eggs.

6. The average size of the ovum of the goat as measured by the inner diameter of the zona is 145·3 μ and the zona pellucida has an average thickness of 12·5 μ.

7. Up to the time of formation of the blastocyst cavity the eggs do not change appreciably in size.

8. Fertile goat eggs enter the uterus sometime between the close of the fourth day and the beginning of the fifth day after mating; that is, at the time when the eggs have acquired their distinctive polarity.

9. The goat is compared with the sheep, the cow and the pig as to the rate of cleavage as far as the blastocyst.

10. In the fixed and sectioned unsegmented egg there is a more deeply staining cytoplasmic crescent around a more lightly staining central mass.

11. The first cleavage spindle was found in an egg at 30½ hr. post-coitum, and the process of division was observed at the same time in another egg of the same litter.

12. The two blastomeres resulting from the first cleavage are of unequal size.

13. The second cleavage takes place at 48 hr. post-coitum, and the spindles in the blastomeres are at right angles to each other, so that at the four-cell stage the blastomeres lie cross-wise.

14. Four-cell stages were recovered from the tube 60 hr. post-coitum.

15. The third and fourth cleavages are slow and require from 12 to 24 hr., the eight- and twelve-cell stages being found at 85 and 98 hr. post-coitum.

16. At the thirteen-cell stage, which is reached about 98 hr. after mating, one blastomere is centrally placed.

17. In the late morula, the commencing formation of the blastocyst cavity is indicated by the increase in size of the intercellular spaces between the centrally placed cells and the smaller trophoblast cells at one pole of the egg. Larger (formative) cells are found at the opposite pole.

18. In the early blastocyst (i.e. during the sixth day) the two portions of the egg, trophoblastic and formative, are completely differentiated. The blastocyst cavity is situated among the smaller trophoblast cells which are capped by the larger formative cells.

19. The trophoblast cells which roof the incipient cavity and are in contact with the deep surface of the formative cells participate in the formation of the entoderm.

20. By the close of the seventh day the blastocyst has attained its chief characteristic, that of a spherical vesicle distended with fluid. Four types of cells are recognizable, formative cells, primitive entoderm cells, covering trophoblast, and parietal trophoblast.

It is with pleasure that the authors record their thanks to the many helpers who have willingly given their services in different ways, and especially are thanks due to the following members of the Departments of Histology and Biology, Royal Veterinary College, where the work was carried out.

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EXPLANATION OF PLATES

All the photomicrographs are reproduced without retouching

Plate 1

Fig. 1. A living unsegmented egg (no. 4B) at 30½ hr. post-coitum, illuminated with incident lighting. ×250.
Fig. 2. The same egg as that shown in fig. 1, illuminated with transmitted light. ×250.
Fig. 3. A uterine egg (no. 14A) at 140 hr. post-coitum, illuminated with transmitted light. Observe the extensive perivitelline space and the greater opacity of the vitellus as compared with fig. 2. ×250.
Fig. 4. A living two-cell egg (no. 1A) at 48 hr. post-coitum, illuminated with transmitted light and showing unequal blastomeres. ×250.
Figs. 5, 6. A living two-cell egg (no. 4A) at 30½ hr. post-coitum obtained from the same fallopian tube as the one-cell stage shown in fig. 1. Fig. 5, illuminated with incident lighting and fig. 6, with transmitted light. Observe the polar body in fig. 6. ×250.
Figs. 7, 8. Living four-cell eggs (nos. 5A, 5B) at 60 hr. post-coitum, obtained from the same female but from opposite tubes and illuminated with transmitted light. Observe the cross arrangement of the blastomeres and the large fat globules in one of the cells. ×250.
Fig. 9. The same egg as that shown in fig. 8 illuminated with incident lighting. ×250.
Fig. 10. A living eight-cell egg (no. 2B) at 85 hr. post-coitum, illuminated with transmitted light. ×250.
Fig. 11. A nine-cell uterine egg (no. 14B) at 140 hr. post-coitum, from the same uterine horn as the one-cell stage shown in fig. 3. Illuminated with transmitted light and showing differences in the size of the cells. ×250.
Fig. 12. A living twelve-cell egg (no. 12A) at 98 hr. post-coitum, illuminated with transmitted light. Observe the differences in size of the blastomeres. ×250.

Plate 2

Fig. 13. A living thirteen-cell stage (no. 12C) at 98 hr. post-coitum, from the same female, but the opposite tube as the twelve-cell stage shown in fig. 12. The smaller cells are compactly arranged and a polar body is seen in the upper part of the figure. ×250.
Fig. 14. A living uterine morula of about thirty cells (no. 7B) at 120 hr. post-coitum, illuminated with transmitted light. Observe the semi-compact arrangement of the cells and the extensive perivitelline space. ×250.
Fig. 15. A living morula of thirty cells (no. 7C) at 120 hr. post-coitum, from the same female as no. 7B, fig. 14, but from the tubo-uterine junction. Observe the loosely arranged blastomeres which form groups of larger and smaller cells at opposite poles of the egg. Note the polar body in the upper part of the figure. ×250.
Fig. 16. A living uterine morula of thirty-two cells (no. 7A) from the same horn as the morula shown in fig. 14. Observe the compact arrangement of the trophoblast cells and the loosely arranged larger formative cells. ×250.
Figs. 17, 18. Two living early uterine blastocysts (nos. 13C, 13B) from the same horn at 134 hr. post-coitum, illuminated with transmitted light. Observe the clear-cut demarcation between the larger loosely arranged formative cells and the compact trophoblastic cells surrounding the incipient blastocyst cavity. Note the complete correspondence of the two eggs. ×250.

PLATE 2 (*continued*)

Fig. 19. The living blastocyst (no. 8) at 156 hr. post-coitum, illuminated with transmitted light and photographed at the equator. The embryonic disc is separated by the large blastocyst cavity from the flattened parietal trophoblast. ×250.

Figs. 20, 21. The same blastocyst as that shown in fig. 19, but photographed with transmitted light at a greater depth (fig. 20) and near the surface (fig. 21). ×200.

Figs. 22, 23. Egg 4B, at 30½ hr. Two consecutive sections (1–2–1 and 1–2–2) through the egg, showing the first spindle cut longitudinally. ×500.

Fig. 24. Egg 14A. Section 1–1–14 (×500). Observe the large fat lacunae in the degenerate vitellus.

PLATE 3

Figs. 25, 26, 27. Three consecutive sections (1–3–20, 2–1–1 and 2–1–2) (×500) through egg no. 4A. Two-cell stage at 30½ hr. Observe the polar body in fig. 27 and the darkly stained cytoplasmic crescent in each of the blastomeres.

Figs. 28, 29. Egg no. 1A. Two-cell stage at 48 hr. Two consecutive sections 1–3–5 and 1–3–4 (×500). In fig. 28 the chromosomes are cut transversely in the larger cell, while in fig. 29 they are cut longitudinally in the smaller cell.

Fig. 30. Egg no. 5A at 60 hr. Four-cell stage. Section 1–4–20 (×500). Two cells only are visible, one, the smaller is in metaphase.

Fig. 31. Egg no. 5B. Four-cell stage at 60 hr. Section 1–4–2 (×500), showing three of the four cells.

Figs. 32, 33. Egg no. 2A at 85 hr., at the eight-cell stage (sections 1–2–2 and 1–1–20) (×500). Five cells are visible in fig. 32; three are distinctly shown in fig. 33. The majority of the nuclei are lobed.

Fig. 34. Egg no. 2B at 85 hr. Eight-cell stage (section 1–1–22) (×500). Four cells are visible. None of the nuclei are lobed.

Figs. 35, 36. Two sections (1–1–11 and 1–1–14) of egg 14B at 140 hr. (×500). In fig. 35 the two non-nucleated cytoplasmic bodies are shown in the upper half of the figure.

PLATE 4

Figs. 37, 38. Two sections (1–1–14 and 1–1–18) (×500) of egg no. 12B. Ten-cell stage at 98 hr. Five cells are shown in each of the figures. Observe the distribution of the fat lacunae, in each of the sections, the polar body in fig. 37 and the irregular nuclei in fig. 38.

Figs. 39, 40. Egg no. 12A at 98 hr. Twelve-cell stage (sections 2–1–6 and 2–1–9) (×500). Eight cells are visible. Nuclei resting.

Fig. 41. Egg no. 12C at 98 hr. Thirteen-cell stage (section 1–3–16) (×500). Seven cells only are shown in the photograph, one of which is central.

Figs. 42, 43, 44. Three consecutive sections (1–2–14 to 1–2–16) (×500) through morula no. 7C with thirty cells at 120 hr. Four central cells of different sizes are shown. Some of the peripheral cells are in mitosis.

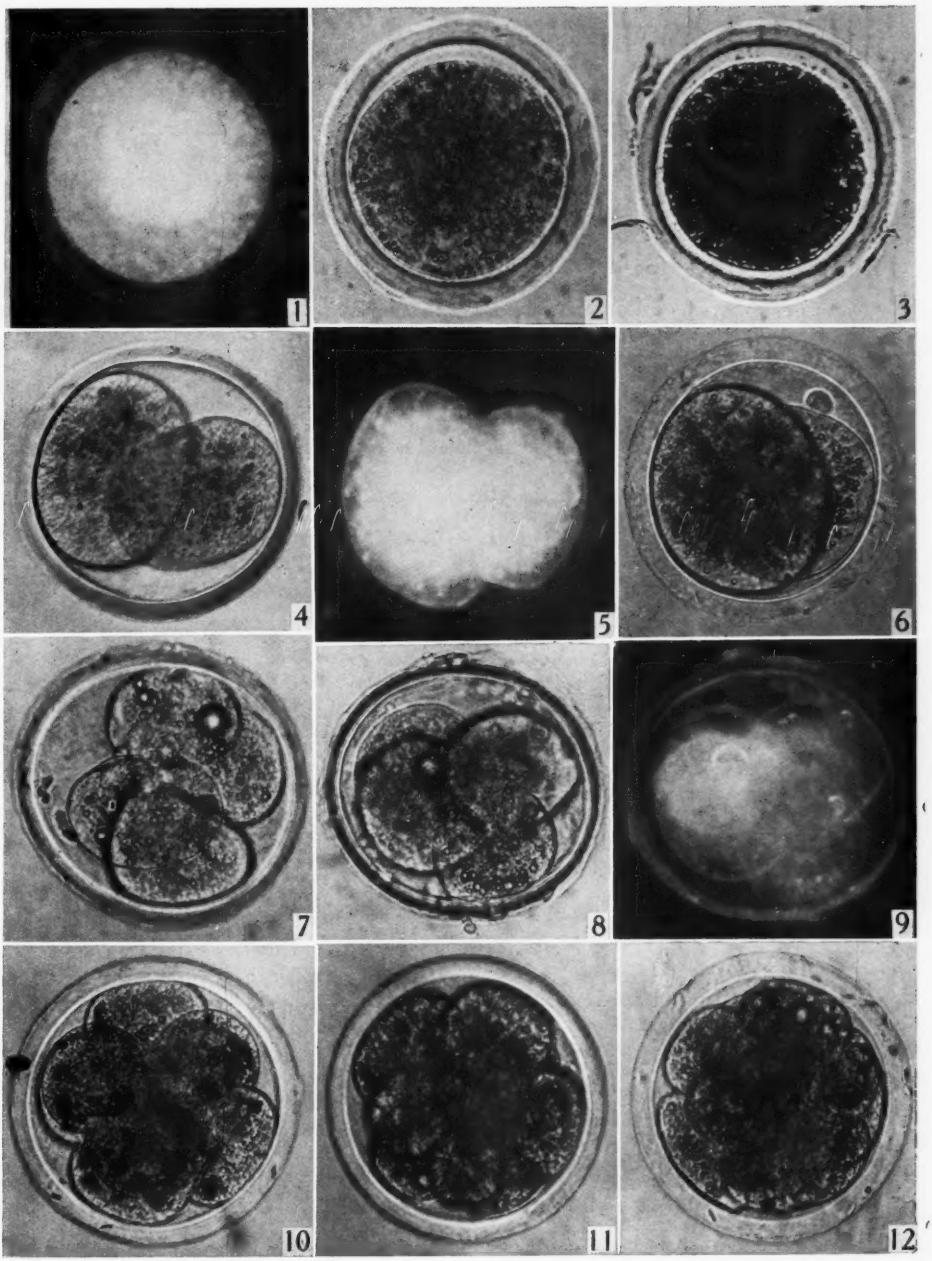
Figs. 45, 46. Morula 7A with thirty-two cells at 120 hr. Sections 1–2–9 and 1–2–10 (×500). The large central cell is in mitosis and is separated by wide intercellular spaces from the peripheral flattened trophoblast cells. Observe the polar body in fig. 46.

Figs. 47, 48. Two consecutive sections 1–2–4 and 1–2–5 (×500), through the opposite pole of the morula shown in figs. 45 and 46. The central cells are smaller and have resting nuclei. Wide intercellular spaces separate the cells.

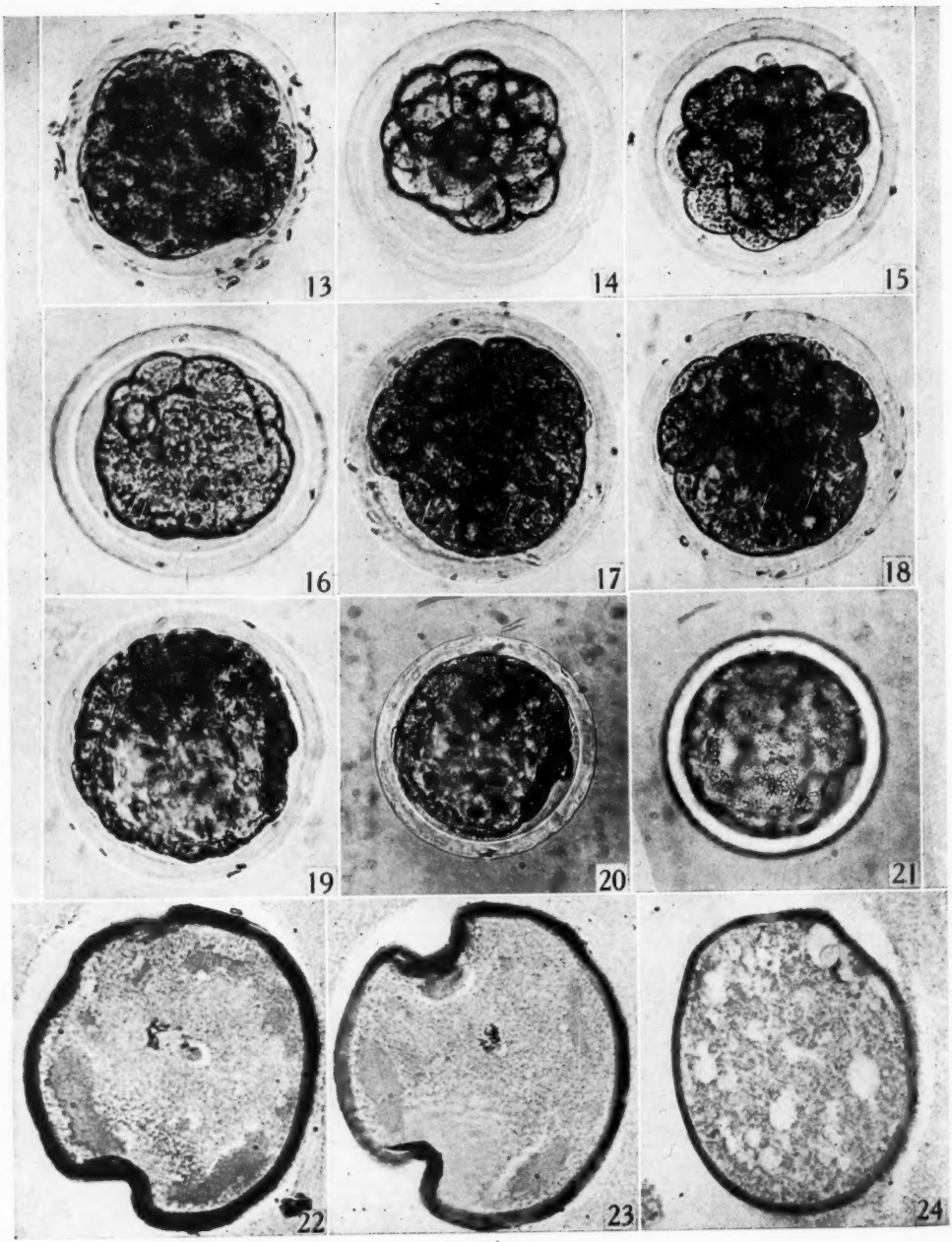
PLATE 5

Figs. 49–52. Early blastocyst (no. 13B) at 134 hr. Four consecutive sections 1–1–17 to 1–1–20 (×500). The demarcation between the trophoblast and the residual formative cells is complete. The blastocyst cavity is represented by a series of intercellular clefts in the trophoblastic portion of the egg.

Figs. 53–60. Blastocyst (no. 8) at 156 hr. Sections 1–2–9 to 1–2–16 (×500). The formative mass is now almost completely enclosed by covering trophoblast on its outer surface and primitive entoderm on its inner surface. Parietal trophoblast cells surround the blastocyst cavity below and on the sides.



AMOROSO, GRIFFITHS AND HAMILTON—EARLY DEVELOPMENT OF GOAT



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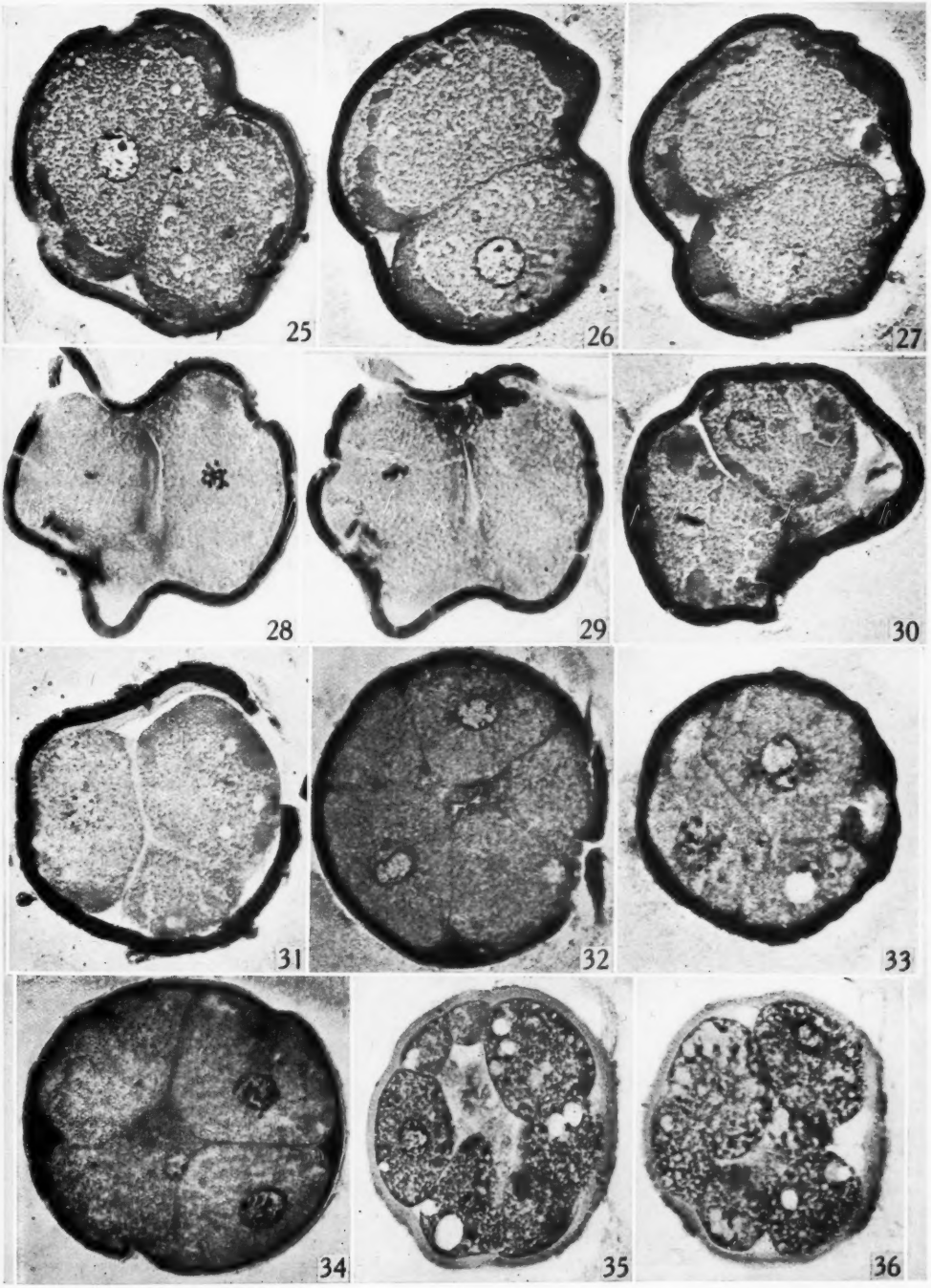

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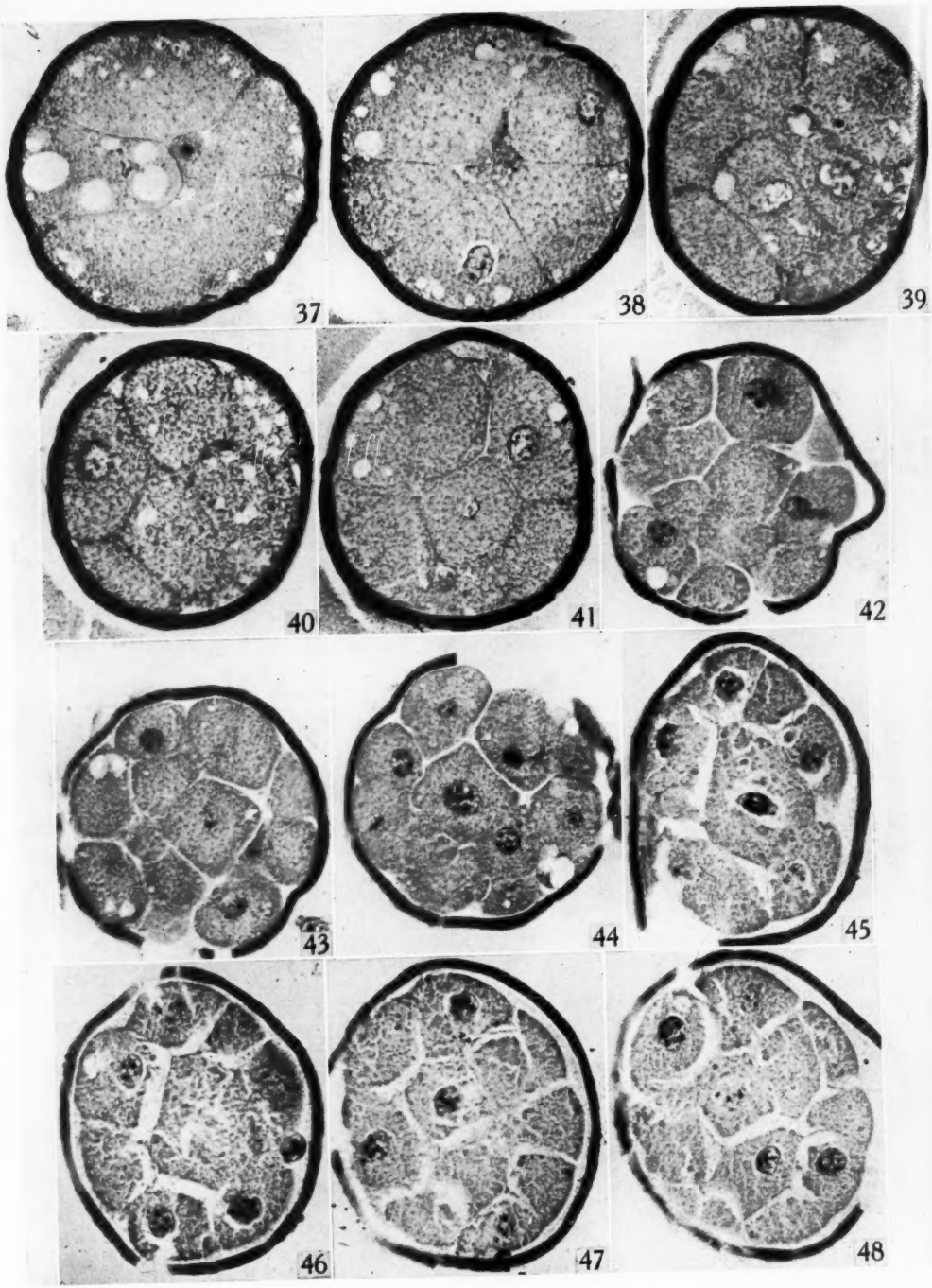
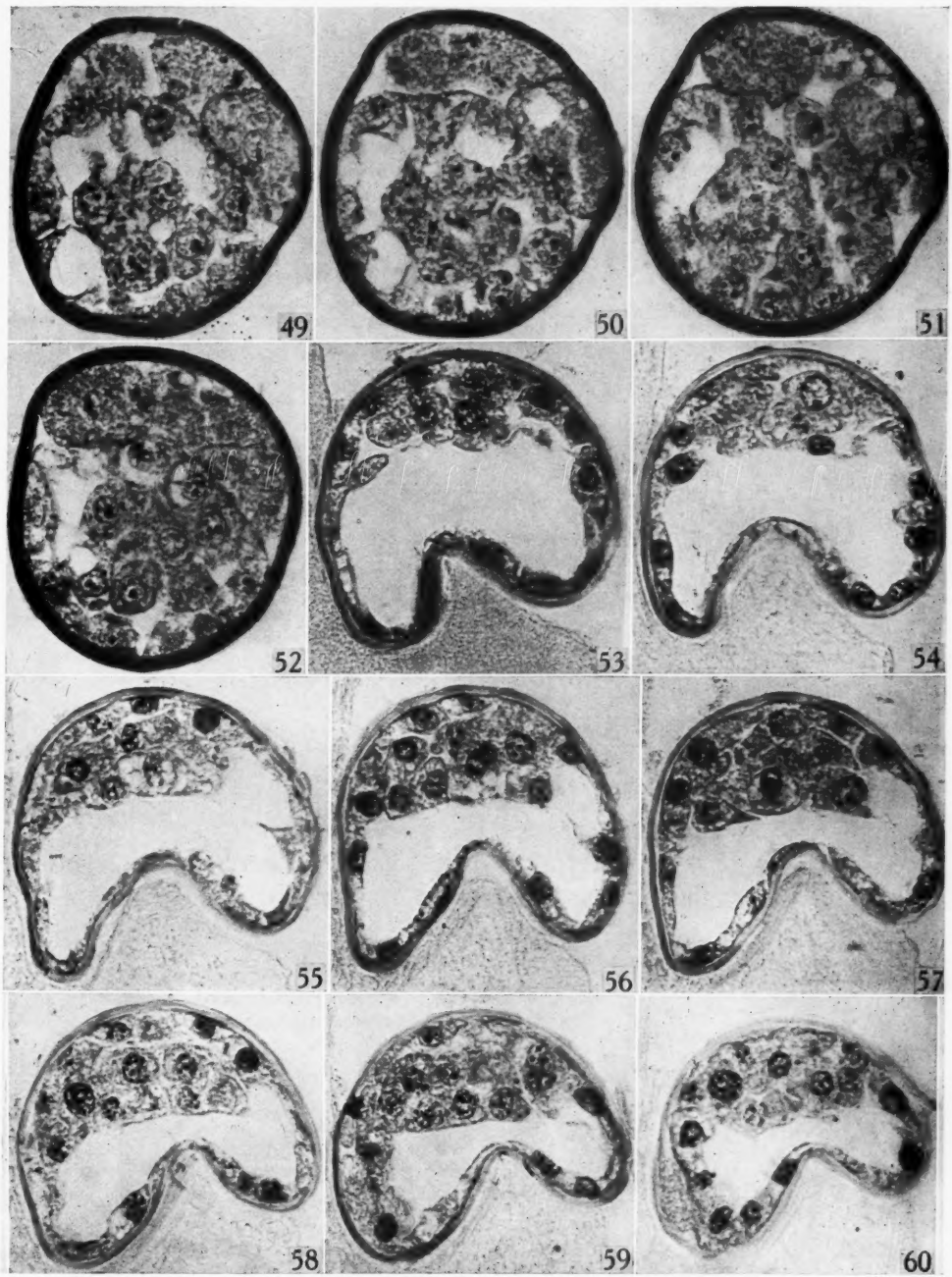


AMOROSO, GRIFFITHS AND HAMILTON—EARLY DEVELOPMENT OF GOAT



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REVIEWS

The Principles of Anatomy as seen in the Hand. By FREDERIC WOOD JONES. Second edition. Pp. x + 417, 144 illustrations. London: Baillière, Tindall and Cox. 1941. Price 25*s*.

Since the appearance of the first edition of this book in 1920, successive generations of the better students have escaped to it with joy from the dry, dispassionate and prescribed texts. In it they found an engaging style and a charm in the illustrations which, when coupled with the comparative approach (always welcomed by students) and the forthright iconoclasm so characteristic of it, provided a welcome and helpful stimulus in what T. H. Huxley called 'that long and weary process of initiation indispensable to the neophyte who aspires to become an adept and to feel at home in the arcana of the higher anatomy'. That a book with such attributes should have become out of print and difficult to obtain is not surprising, and it seems quite certain that the second edition will meet with a demand. The changes from the first edition are numerous: there are six new chapters, nearly a hundred more pages of text, twenty new figures, and the whole book has been revised. Unfortunately, the two plates of the original edition, and which are still referred to in the text, are now absent, which is a great pity, as one of them, the frontispiece, was a reproduction of Rembrandt's 'Anatomy Lesson' and must have given pleasure to many readers of the first edition.

In reviewing a book such as this it is impossible to avoid issues raised both by its title and its contents concerning the position and role of anatomy in the medical curriculum. Explicitly and implicitly Prof. Wood Jones's statements show that he is dissatisfied with formal topographical anatomy which emphasizes details and which is not concerned with broad principles. Topographical text-books are, of course, a necessity for reference purposes and cannot be supplanted—but the student must be taught to use them as reference texts should be used. This implies that they must be supplemented by books which, in attitude and approach, provide the student with a survey of the general biological principles of anatomy, make him interested in observing for himself what is significant, and help him to escape from the thrall of the authoritative text. The book under review performs all of these functions and is a very useful contribution to the small group of supplementary texts which bridge the gap between the reference book and the original literature, and which make the reader realize that anatomy is a living and growing subject.

In the reviewer's opinion, however, there are several features which tend to detract from its value. In the first place, it can only be read with complete profit by a senior student with a well-developed critical faculty. This is partly due to the approach which, being morphological, entails a knowledge of comparative anatomy unusual outside the ranks of academic anatomists and zoologists. Further, the morphological interpretations are occasionally used to imply the acceptability of certain hypotheses which are repudiated by most biologists and, as Prof. Wood Jones is a skilled dialectician, continual attention is required to avoid being too easily persuaded by his subtle and ingenious arguments. Thus, on more than one occasion he suggests that new structures are developed as a direct response to the demands of function, and he interprets the loss of structures as the direct result of their having become undesirable. The reviewer believes that such a teleological approach to the problems of evolution is sterile. If the history of biological thought in the past forty years (in fact since Bateson, de Vries, Morgan and their successors changed the whole approach to evolutionary problems) has taught any lesson, it is that morphology alone is not a sufficiently adequate discipline in the study of phylogenetic change. It must be supplemented by genetics and experimental embryology, and he who would treat of evolution seriously must consider the results of both of these subjects. In all the matter in Prof. Wood Jones's book dealing with evolution genetics is never mentioned. It can, of course, be argued that the science of genetics constitutes a special subject, and in detail it is a rigorous and difficult one, but its general story is of such transcendent importance to anatomists, embryologists and clinicians that it deserves a place in the principles of anatomy. Its absence, and the

suggestion of neo-Larmackianism which tinges so many of Prof. Wood Jones's statements, somewhat limit the value of his book. One can go further and suggest that references to Goldschmidt, Dobzhansky, Haldane, and Needham would prove more useful and be more in temper with our mental climate than references to Paley and the Bridgewater Treatises, which cannot seriously be maintained to have more than an antiquarian interest.

In another field, too, Prof. Wood Jones has not incorporated the results of modern investigation. The work of Head and Rivers which resulted in the conception of protopathic and epicritic sensation will always mark a definite step forward in our knowledge of peripheral sensation. But it is a conception that can no longer be maintained. The work of Trotter and Davies, Boring, Schafer, Lanier and, most recently, Woollard and Weddell, provides an alternative explanation of all the phenomena which resulted in Head's interpretation, and no good purpose is fulfilled by retaining, as Prof. Wood Jones does, an *ad hoc* hypothesis which subsequent research has rendered unnecessary and which, moreover, is confusing to the student.

On the other hand, many portions of the book are excellent. The description of the results of peripheral nerve lesions and the explanation of the anatomical basis for the deformities in peripheral palsies are more convincing than any the reviewer has met. This holds, too, for the action of muscles in general, and all students can learn much from the clear and reasonable presentation of what has always been a thorny subject. Fasciae are described in excellent perspective and to the confusion of those who overstress them, and the treatment of the vascular system shows how much can be taught to a student without petty detail.

Prof. Wood Jones's book, like all his contributions, is highly individual and it is not a ways easy to know when principles and when details (however important) are being considered. It is obviously impossible for a biologist, and especially for a morphologist, to give a full exposition of principles in the present state of our knowledge, and the title should perhaps better read '*some* principles of anatomy'. But the book is not an 'anarchy of generalities', and, although some important principles are completely absent, those which are expressly stated and others inherent in the treatment make it and its contents worth possessing. Any student will be the better anatomist for having read it.

J. D. B.

Functional Neuroanatomy. By W. J. S. Krieg, Ph.D. Pp. 552, figs. 273. (Published by the Blakiston Company, Philadelphia.)

The outstanding feature of this book lies in the originality and ingenuity of the illustrations. Indeed, it is stated in the preface that they form 'the life-blood of the work'. They are the work of the author himself, and it is clear that he has enjoyed to the full the abundant opportunities for draughtsmanship which a new text-book on neurological anatomy provides. Many of the illustrations are in the nature of plastic diagrams representing transparent outlines of various parts of the brain, with tracts and nuclei faintly indicated in their relative positions within—a type of diagram which is commonly employed in continental text-books. Some of these illustrations are attractive and illuminating, and all have evidently been executed with great care and forethought. Yet, the reviewer feels that many of them fail to achieve their object, because they are too complicated. The whole function of a diagram is surely to reduce a set of relations to such simplicity that their fundamental arrangement at once strikes the eye. The more a diagram tries to imitate reality, the less effective does it become. For example, the features illustrated in Figs. 131, 136, 141, 150, 162 (to mention only a few) would be much more clearly demonstrated by the usual two-dimensional diagram. Again, the three-dimensional representations of the structure of the retina and of the cerebellar cortex are definitely confusing to the eye, and cannot possibly be so useful to the student as the time-honoured sectional diagram. The reviewer feels, also, that this book is rather too detailed for the ordinary medical student, and at the same time does not adequately fulfil the requirements of the advanced student of neurology. We note that the author decided in preparing his book to introduce neither the opinions nor the names of individual authors who have contributed to our knowledge of the nervous system. The result is that some statements are made which are apparently authoritative but which in fact rest on controversial evidence, and it may reason-

ably be argued that an advanced student cannot really be given a balanced view of the subject unless he is made acquainted with sources of evidence and the possibilities of alternative interpretations.

Yet the book is certainly to be recommended to those who have already completed some neurological training, for it is written in an attractive style and provides a useful résumé of present-day knowledge of neuroanatomy. It may be remarked, also, that the volume has been most attractively produced. Indeed, with its end-paper decorations and its illuminated capital letters, it may well be called an 'edition de luxe'. We note, by the way, that in a list at the end of the book of periodical publications in English which contain articles on neuroanatomy, no mention is made of the *Journal of Anatomy*. This omission strikes us as odd.

Lymphatics, Lymph and Lymphoid Tissue. By C. K. Drinker and J. M. Yoffey. Pp. 406. 1941. (Harvard University Press.)

In normal times, new books dealing with anatomical and physiological matters are published with fair frequency, and yet it must be admitted that perhaps the majority of them have an evanescent career. Every now and again, however, there appears a monograph of quite outstanding importance which is clearly destined to find a secure place in the reference library of those concerned with the same or allied fields of work. Such a monograph is the book on the lymphatic system which has recently appeared under the authorship of Drinker and Yoffey. Here will be found an unusually comprehensive and up-to-date study of a subject of supreme interest to anatomists, physiologists and clinicians. Based partly on the well-known systematic researches of the authors, it comprises an extensive and scholarly review of the observations of all those workers who have made serious contributions to the elucidation of the varied problems of the lymphatic system. This survey covers the organization of the lymphatic apparatus, the intricate subject of the permeability of blood and lymph capillaries and the factors on which this depends, the various conditions affecting lymph flow, the chemical and physical properties of lymph, the biological significance of lymphoid tissue, and all the studies concerning that mysterious entity, the lymphocyte. Not the least important aspect of the work is that it crystallizes very succinctly the immediate problems that now face the anatomist and physiologist who are engaged in the study of the lymphatic system, and upon the solution of which depend the next steps in the advance of our knowledge concerning it.

It is a matter of some interest to note that this book is the product of a collaboration between a Professor of Physiology and a Professor of Anatomy. It is, indeed, yet another expression of the fruitful results which must necessarily ensue with the obliteration of the artificial barrier between anatomy and physiology—a barrier which even in the recent past has had rather a sterilizing influence. Prof. Yoffey's contribution is also a significant indication of the changing attitude in this country of the anatomist to the subject of his profession. The phase of anatomical science is rapidly passing when the anatomist was content with the description of static structural relations, seeking to 'explain' them entirely on the basis of untested hypotheses. On the contrary, the necessity of putting hypotheses to the test by experimental enquiry is now recognized to be an essential requirement for real progress in anatomy as it is also in other biological sciences. By his own contribution to the investigation of the lymphatic system, Prof. Yoffey has indicated how much can be achieved by such an approach. His discussion of the biological significance of lymphoid tissue also suggests by implication how the subject of the lymphatic system should be presented rationally to the student of anatomy. Show a student a single preparation of a lymph node from an animal in which, during life, the afferent lymphatic vessels have been injected with a suspension of india ink (as illustrated in Fig. 31 of the book under review), and he will get more understanding of the fundamental anatomy of the lymphatic system than he will from any number of blackboard diagrams representing arbitrary groups of nodes interconnected by directing arrows—pictures which are so commonly memorized for examination purposes by students but which commonly bear no relation whatever to reality.

The authors of this book are to be congratulated on a very successful achievement—the book will certainly be the standard work on the lymphatic system for some time to come.

Bibliographia Primatologica. A Classified Bibliography of Primates other than Man. Part I. By THEODORE C. RUCH. Pp. xxviii, 241. Price 47*s*. (Baillière, Tindall & Cox, 7 & 8 Henrietta Street, London W.C. 1.)

Scientists have always owed a great debt of gratitude to those of their comrades who undertake the very laborious task of compiling bibliographies. Dr Ruch's bibliography is particularly welcome, since it deals with a literature which is unusually scattered. Moreover, with the modern trend of experimental work related to the functions and dysfunctions of our own species, the primates as a group now assume more and more importance.

The present volume deals with the anatomy, embryology, histology, physiology, pharmacology and experimental psychology of the extant primates. As Professor Fulton points out in his introduction, it is a matter of some surprise that the literature of the primates is not larger—the number of authors who have studied the modern primates from the point of view of anatomy, physiology and behaviour is little more than two thousand, and the total number of papers dealing in whole and in part with these subjects does not reach five thousand. It is intended that Part II of this work will include the literature of the pathological sciences (pathology, bacteriology, immunology and parasitology). We would plead, also, for a part dealing with fossil primates, of which the literature is possibly even more extensive than that of recent forms.

It is almost unnecessary to say that Dr Ruch's bibliography is quite essential for any scientist whose work involves the study of primates other than man, and we can certainly warmly recommend it for the clarity of its style and form.

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